The Effects of Sensory Stimulation and Arousal on Stepping in Newborns

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Abstract

Kim Siekerman - The Effects of Sensory Stimulation and Arousal on Stepping in Newborns

Humans produce step-like movements from birth. Stepping has been successfully utilised in treadmill training for infants at risk of developmental delay. Previous studies examining optimal training contexts focused on infants from one month of age: a knowledge gap exists for newborn stepping. This thesis explored stepping in three-day-old newborns and investigated whether tactile-proprioceptive and visual stimuli can improve stepping, whilst also controlling for and examining the effects of arousal. Experiment 1 examined if treadmill stimulation alone would improve stepping. Twenty-one newborns were supported, in four one-minute sessions, on a static or moving treadmill. In Experiment 2, 20 newborns were supported over a friction-enhanced treadmill with four different optic flow conditions: no optic flow, optic flow moving congruent or faster than the treadmill, or in random directions. Video footage, three-dimensional kinematic data (Experiment 2) and electromyography were recorded. Treadmill movement stimulated forward steps but not vertical flexion-extension cycles. Cycle durations and muscle burst lengths decreased on the moving treadmill, without showing a linear relationship with treadmill speed. Optic flow on the moving treadmill did not affect step rate or coordination, although random optic flow increased pump rate, inter-joint coupling and hip extension. Fastest optic flow deactivated muscles during the stance phase. Arousal improved step rate, swing and stance definition and interlimb alternation in Experiment 1. In Experiment 2, arousal increased hip extension, inter-joint coupling and muscle activation ratio, without changing temporal and spatial muscle patterns. Improved belt friction may have caused the differences between experiments. In summary, newborns tolerated stepping on the treadmill and with optic flow. Step rate, coordination, kinematics and neuromuscular behaviour were highly variable but could be modified by tactile-proprioceptive and visual stimulation. Arousal universally stimulated stepping, likely through increased muscular activity. Findings support the use of treadmill training from birth, although long term effects require further investigation.
Author’s declaration

I hereby declare that the work contained within this thesis is my own work other than the counsel of my supervisors, Professor Alan Donnelly, University of Limerick and Dr. Caroline Teulier, Université Paris-Sud, and of collaborative work completed with Dr. Marianne Barbu-Roth, Université Paris-Descartes. This work has not been submitted for any academic award, at this or any other educational establishment. Where the use has been made of the work of other people, it has been fully acknowledged and referenced (bibliography).

________________________
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List of abbreviations

2D: Two-dimensional
3D: Three-dimensional
AR: Activity ratio
CPG: Central pattern generator
DOF: Degrees of freedom
DV: Digital video
EMG: Electromyography (sEMG stands for surface electromyography)
GC: General coactivity
MBL: Muscle burst length
MU: Movement unit
OF: Optic flow
POA: Probability of activation
QTM: Qualisys Track Manager
RM ANOVA: Repeated measures analysis of variance
ROM: Range of motion
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Chapter 1: INTRODUCTION
1.1 Background

Neonatal stepping has been researched extensively with the aim of understanding the processes that underlay the development of independent walking. Once an infant learns to walk without assistance, he or she will be able to explore the world more freely than if crawling or bum-shuffling was their main mode of transport. A period of rapid development of cognitive, spatial, emotional and further motor development commences. Because walking is such an important skill, it is important to understand the ontogeny of its possible precursors: neonatal stepping is one of them. Different theoretical perspectives have led to diverse interpretations of the regulation and development of stepping. One view is that neonatal stepping is controlled far down the brain hierarchy, in special neural networks in the spinal cord (Yang et al., 1998a, Yang and Gorassini, 2006) and is distinct from adult walking, which is organised in the motor cortex. According to this view, neonatal stepping is inhibited when the higher brain takes over control of movement, although some researchers propose that the spinal patterns are retained as building blocks of mature locomotor activity (Dominici et al., 2011). Others proposed that all movements are a product of many different external factors and internal processes (Thelen and Ulrich, 1991, Thelen and Cooke, 1987), and that stepping is expressed in various forms in the newborn period – i.e., swimming, kicking, crawling and air stepping. It may be that the term ‘stepping’ is over-specific - and views the movement too much from a mature locomotion perspective (Campos et al., 2008) - whereas the naturally occurring movement only resembles walking when infants are constrained in an upright position. Additionally, the movement behaviour does not disappear but is suppressed by an unfavourable leg strength-to-weight ratio in its upright form; it can still be observed in facilitating environments, like in water or on a motorised treadmill, or when lying supine (Thelen et al., 1984, Thelen, 1986b). They also interpret stepping as being within a developmental continuum with walking, and have shown that it can be trained (Vereijken and Thelen, 1997).

It is essential to understand the differences between these viewpoints, because they attribute an entirely different role to stepping. The dependence of maturation on the cortex and corticospinal pathways suggest that there is little reason for eliciting stepping in infants beyond studying its forms in research. Conversely, dynamical systems theory views that development is a process where experience influences maturation, and, reciprocally, the level of maturation affords new behaviours. The great level of plasticity and trainability of stepping led to its use in a clinical application. Ulrich and colleagues
presented a training paradigm that helped infants with Down syndrome attain an earlier onset and a greater proficiency of independent walking (2001). Because walking is a major motor milestone, earlier walking in at-risk children may help limit delays in other areas (Smith and Bompiani, 2013). Importantly, early and aggressive rehabilitation is preferred to attempting to remedy existing motor delays and the associated physical problems (Ulrich, 2010). The effectiveness of Ulrich’s training study precipitated further experiments investigating role of various sensory pathways to stepping. If stepping was linked to independent walking and could be trained, what were the most effective and appropriate ways to elicit stepping?

Stimulating stepping through augmenting the training environment with a tactile-proprioceptive stimulus (treadmill) has shown clear benefits. Infants with Down syndrome walked on average 101 days earlier than their untrained peers when they were supported over a moving treadmill (Ulrich et al., 2001). There was a significant dose response, with high intensity, individualised treadmill training giving better results in terms of walking kinematics than low intensity, general treadmill training (Ulrich et al., 2008, Wu et al., 2010). This improvement was even observable a year after the training program took place. High intensity training was also associated with attaining some motor milestones at an earlier age (Ulrich et al., 2008). Clearly, tactile-proprioceptive stimuli through the treadmill improves stepping in infants with Down syndrome (Ulrich et al., 2001) and typical development (Thelen and Vereijken 1997).

Additionally, the visual system has been discovered as a precocious system that is involved in locomotion (Barbu-Roth et al., 2009) and posture (Jouen et al., 2000) from birth. Three-day-old newborns responded with increased air stepping when supported over a surface with an optic flow projected on it when it mimicked forward motion (Barbu-Roth et al., 2009). Infants from two months of age stepped more when a visual pattern was added to the treadmill belt (Moerchen and Saeed, 2012), with stronger effects for older infants (Pantall et al., 2011, Pantall et al., 2012). Infants from about two months of age appear to respond positively to augmentation of the sensory context of the training environment.

However, some knowledge gaps still exist. Firstly, there are few studies that examine stepping in its different forms directly after birth (Barbu-Roth et al., 2009, Barbu-Roth et al., 2013, Barbu-Roth et al., 2015, Domellöf et al., 2007, Dominici et al., 2011). None of these studies examined newborns on the treadmill directly after birth and therefore it is not known if treadmill stimulation has the same benefits during the newborn period as for
later infant stepping. It is also not clear if a combination of dynamic tactile-proprioceptive stimulation (moving treadmill) combined with optic flow will give the same benefits in newborns as in their older peers (Teulier et al., 2012, Moerchen and Saeed, 2012). Being able to start treadmill training from birth may have benefits, as this would allow an increased amount of training to take place during a time of great neuromuscular plasticity (Ulrich, 2010). Furthermore, the role of arousal on the quantity and quality of movement in infants is not well studied. It is important to ensure that newborns are not over-aroused, which may suggest that they do not tolerate the testing well. However, it was also found that stepping was more easily evoked when infants were at higher arousal states, (Thelen et al., 1984), and, reciprocally, that movement may increase arousal (Thelen, 1986b). Furthermore, it is important to ensure that any effects from sensory stimulation are not caused by an increased arousal level in response to the stimulus. It is therefore essential to monitor arousal in this population group. It is known that arousal generally facilitates stepping, but it may also affect stepping at the neuromuscular level or affect coordination. Finally, the characteristics of newborn stepping have only been described on a very basic level; few studies looked at kinematics or neuromuscular behaviour. An analysis comparing the results of different levels of analysis is needed to extend the current knowledge base of newborn stepping.

1.2 Aims and research questions

The lack of detailed data on treadmill stepping in the youngest newborns (three days old) prompted the first aim in this project: to ascertain that newborns are able to endure short periods of supported treadmill stepping directly after birth. A significant increase of arousal during testing may be interpreted as poor tolerance of the procedure although it could also indicate a general arousing effect of upright stepping. Additionally, if increased arousal was strongly associated with a sensory stimulus, it would be difficult to parse its influence from that of the stimulus. Detailed mapping of arousal on steps may clarify these relationships. The detailed recording of arousal could also shed further light on the effects of arousal on movement characteristics.

Further, this thesis aimed to ascertain whether tactile-proprioceptive (treadmill) or a combination of tactile-proprioceptive and visual (optic flow) stimulation had similar benefits to stepping characteristics as it does in older infants, where it advances step rate and/or coordination. If newborns show a positive response to the treadmill and/or the
optic flow enhanced treadmill it may be possible to start treadmill training directly after birth, although longitudinal studies would have to confirm its efficacy. There may be benefits to training stepping when it is still easily elicited and in a highly responsive and variable transition period, and it is important not to lose any time when it comes to countering the effects of developmental delay. Additionally, it would offer further support for the complexity of the control mechanisms of stepping in the newborn. Thus, this thesis will address the following research questions:

- Do newborns respond to a moving treadmill with increased stepping?
- Do they adapt to treadmill speed in a similar way as their older peers do?
- Is there a quantitative or qualitative benefit to adding optic flow to the moving treadmill, and do changes occur on a kinematic or neuromuscular level?
- How does arousal affect stepping?
- Do the results show evidence of spinal pattern generation or do they support the importance of multiple complex non-linear factors in stepping?

To assess the effects of tactile-proprioceptive and visual stimulation on stepping directly after birth, this project will perform multi-level analyses on treadmill stepping in three day old infants in two experiments. These studies will focus on stepping on a static and a moving treadmill (Experiment 1) and on stepping on a moving treadmill enhanced with optic flow (Experiment 2). In both experiments, arousal will be monitored in detail. The results will be discussed with a view to current theories.

1.3 Thesis structure

The body of this thesis consists of eight chapters. Chapter 1 contains a short introduction of the background, the aims and research questions, and the thesis structure. Chapter 2 consists of a review of the current literature, and a methodology section follows in Chapter 3. Data from two experimental studies are analysed and discussed in the Chapters 4 to 7. These chapters are presented in journal article format, with an introduction, methodology, results and discussion section. The first chapter was published, and the second chapter has been finalised and is awaiting submission. A general discussion (Chapter 8) organises the findings with a view to the research questions, discusses the limitations and offers suggestions for further research. Each thesis chapter is outlined below:
Chapter 2 reviews the literature by outlining a brief history leading to the current knowledge of stepping at birth. It discusses divergent theoretical viewpoints within motor development and the control of the stepping mechanism, and highlights their importance in consideration to clinical intervention and basic science. It covers the current knowledge of the role of context on movement behaviour, and highlights the roles of environmental and organismic influences.

Chapter 3 lists methodological considerations common to both studies, whilst leaving the specific methods for description in each chapter.

Chapter 4 discusses the influence of treadmill stimulation on the quality and quantity of stepping at birth. Additionally, it presents the confounding factor of arousal to infant motor behaviour.

Chapter 5 explores the effects of multi-modal stimuli on stepping at birth. The links between the visual and tactile-proprioceptive system and locomotion are discussed.

Chapter 6 quantifies joint kinematics of stepping in response to optic flow combined with the treadmill. It discusses differences and similarities to the findings in Chapter 5.

Chapter 7 delves one level deeper to describe the neonatal response to treadmill stimuli and visual flow on a neuromuscular level by exploration of the electromyographic output of the muscle. It contrasts these results with those of Chapters 4, 5 and 6.

Chapter 8 discusses the results from Chapter 4 to 7 by amalgamating overtly visible, kinematic and electromyographic findings in response to various external stimuli and internal factors. It will make a case for a new interpretation of neonatal stepping and its importance for early rehabilitation of at-risk infants.
Chapter 2:

REVIEW OF LITERATURE
2.1 Literature review study methods

A literature research was performed by searching the databases Pubmed and Web of Science, with additional searches in Google Scholar and the University of Limerick Library Database. The following search terms were used: “newborn”, “neonatal”, “infant”, “foetus”, “human”, “stepping”, “walking”, “locomotion”, “leg movement”, “treadmill”, “treadmill training”, “optic flow”, “visual stimulation”, “visual system”, “arousal”, “behavioural state”, “kinematics”, “EMG” and “electromyography”, “neuromuscular behaviour” and “biomechanics”. All articles on infant movement behaviour relevant to stepping research were included. Articles featuring toddler, child and/or adult walking behaviour were included if they included a discussion of infant behaviour from a developmental perspective. For the optic flow section, literature on infant vision was limited to optic flow processing, and in the arousal section, all articles in a limited list of resources on arousal were selected, excluding the “arousal from sleep” articles. The aim of the article was to include articles from various theoretical perspectives on a broad range of topics within infant stepping, with a special focus on newborn stepping. A non-structured approach was therefore deemed most suitable, and therefore, further searches were performed throughout the writing to include all the relevant links that were found in the primary articles, particularly when they were required to clarify theoretical aspects.

2.2 Why study neonatal stepping?

Human neonates come into the world with a broad repertoire of complex movements (Hadders-Algra et al., 1993). In utero, foetuses have been reported to twist, squirm, flex and extend their limbs (De Vries et al., 1982), and even make coordinated goal-directed movements (Zoia et al., 2007) and flexion-extension cycles with characteristics of neonatal stepping (De Vries et al., 1985). These movements continue outside the womb, after birth. The different forms of movement can be elicited in different contexts, and are perhaps related to each other. For instance, crawling consists of flexion and extension of the arms and the legs while prone and in contact with a surface (Piek, 2006); kicking features flexion-extension cycles of the legs while supine but without tactile stimulation to the feet (Thelen and Fisher, 1982); air stepping involves similar movements while supported upright or prone in the air without tactile input (Barbu-Roth et al., 2013, Barbu-Roth et al., 2009, Barbu-Roth et al., 2015); and in newborn stepping, infants are supported
upright and receive tactile input from the surface below their feet (Thelen and Fisher, 1982). Stepping has been of particular interest to many researchers since the start of the previous century (McGraw, 1932, Shirley, 1931, McGraw, 1940, McGraw and Breeze, 1941), likely because of its resemblance to adult walking even if the two are not the same: infants need to be supported in order to step in the upright position. Subsequently, links between stepping and independent locomotion were drawn (André-Thomas and Autgaerden, 1966, Zelazo et al., 1972), and the behaviour was utilised in training interventions with infants at risk of delay in walking (Ulrich et al., 2001, Wu et al., 2007). However, after nearly 100 years of work, the interpretation of stepping within the newborn movement repertoire, the locus of its control and its role within the development of locomotion are still a point of discussion today. Particularly because of its training potential, stepping deserves thorough attention. To explain the current viewpoints, the following review will discuss relevant literature on stepping throughout the first year of life - with a focus on the neonatal period - through the perspective of the divergent theories. The effect of context is explored through the discussion of the role of the sensory system, and the effects of changing arousal are highlighted. Further, this chapter will describe the training of stepping as a clinical tool, and knowledge gaps and directions for further research are detailed. Finally, the objectives of this thesis are stated.

2.3 What is neonatal stepping?

The classical definition of neonatal stepping refers to the ability of newborns to perform step-like flexion-extension cycles in response to a tactile stimulus to the foot sole when supported upright. The movements resemble erect adult bipedal locomotion, with a swing phase, in which the foot lifts off the surface and moves forward through the air, and a stance phase, in which the foot is on the surface and the body moves forward respectively to the stance leg (Thelen and Fisher, 1982). Furthermore, newborns are able to alternate stepping between the left and the right leg. However, there are some differences with adult walking, and steps are often described as ‘march-like’ (Thelen, 1986b), with simultaneous hip and knee flexion during the swing phase (Forssberg, 1985). Additionally, hips and knees are kept flexed throughout the movement. There is a distinct lack of forward progression, with no active propulsion during the stance phase, and no forward-placed heel strike during the swing phase. Additionally, newborn stepping involves only limited weight bearing and does not involve control of balance, as newborns are fully supported. Stepping is rhythmical and organised, but movement occurs localised
at the lower limbs and ‘not in functional relation with the head and upper part of the body’ (McGraw, 1940, p. 750) - that is, from an adult walking perspective, where an opposing arm swing balances rotation caused by the leg swing. It should be noted, however, that the manner of holding the infant while stepping may block arm movements that may be functional for newborns. It is not clear why newborns step, although most researchers agree that stepping already occurs in the womb. Some researchers suggest that stepping patterns are ‘non-functional phylogenetically old patterns’ (Peiper, 1963); others think foetuses utilise these movements to position themselves in the womb (Oppenheim, 1981, Robinson, 2005); some place neonatal stepping in a developmental context and see it as a precursor to independent walking (Zelazo et al., 1972), either as a hard-wired neural building block (Dominici et al., 2011) or an emerging contextual movement behaviour (Thelen et al., 1991). It is clear that the origins of human stepping are still a debated topic.

2.3.1 The development of stepping throughout the first year

Infants will typically exhibit a strong stepping response at birth, but its expression gradually decreases and normally disappears when they are approximately two months old (Thelen and Fisher, 1982). After several months of not stepping, a similar, voluntary behaviour returns towards the end of the first year, just before infants are learning to walk independently (Thelen and Fisher, 1982). Classically, the disappearance of stepping is attributed to cortical inhibition (McGraw, 1932, McGraw, 1940). Neonatal stepping was viewed as a primitive reflex, together with reflexes such as the Moro, grasping and sucking reflex (McGraw, 1932). Reflexes are involuntary, automatic responses to specific stimuli that are controlled in the lower central nervous system - the brain stem or the spinal cord. As infants gain voluntary control of motor activity, under the influence of maturation of the higher brain structures - the cortex -, reflex stepping is suppressed or inhibited (McGraw, 1940).

The reflex as an explanation for neonatal stepping lost support in later years, and in its place came the view of stepping as a more complex subcortical process. However, the idea that stepping is suppressed by cortical influences or overlaid by cortical control persists in the neuronal network and central pattern generator theories (Dietz, 2003, Grillner and Wallen, 1985, Yang and Gorassini, 2006, Dominici et al., 2011). Simultaneously, Esther Thelen and her colleagues interpreted the disappearance of stepping from a different point of view. They proposed that neonatal stepping does not
disappear, but continues in a different form: supine kicking (Thelen and Fisher, 1982, Thelen et al., 1984). Upright stepping is suppressed because of an unfavourable strength to weight ratio, which makes vertical leg movement considerably more difficult for infants. This view gave rise to a new theory of infant motor development, which centralises the idea that the development of motor behaviour depends on multiple interacting factors, rather than the maturation of the cortex alone. Currently, both viewpoints exist alongside each other. The following paragraphs detail specific knowledge of stepping during the first year gained through both perspectives.

2.4 Theories of stepping

2.4.1. Origins - neuromaturational theory

With a thorough observation of infant stepping from birth to independent walking, Myrtle McGraw presented the first comprehensive description of stepping (McGraw, 1940). She roughly described the overt kinematics of the phases and gave a frequency profile of at which age infants typically attained the phase. Seven phases were defined: 1) the newborn or reflex stepping phase; 2) the inhibitory or static phase; 3) the transition phase; 4) the deliberate stepping phase; 5) the independent stepping phase; 6) heel-toe progression and 7) the integrated or mature phase of erect locomotion. Based on the knowledge available of structure and function of the human CNS at the time, progression through the phases was attributed to a reorganisation between nuclear – or subcortical – and cortical centers. At birth, the cortex was thought to be inactive; therefore, neonatal stepping began under sub-cortical control. Stepping subsequently disappeared due to inhibiting influences of the maturing cortex, and then re-appeared with evidence of increasing cortical control. Voluntary independent walking was controlled exclusively by higher brain centers (McGraw, 1932, McGraw, 1940). Although McGraw believed that function followed the maturation of structure, she also suggested that functional processes may affect structural development of components (Heriza, 1991). Additionally, she acknowledged individual variations and attributed them to differences in temporal order of advancement of functions (McGraw, 1940).

McGraw was the first to place stepping within a developmental trajectory which commenced with reflexive stepping at birth. Later research suggested that control of stepping in newborns was more complex than a simple reflexive stimulus response. For instance, electromyographic (EMG) studies found that extensor activity began prior to
foot contact in the stance, which indicated that muscle activity was ‘not triggered by the contact’ (Forssberg, 1985). The discovery of complex spinal neural networks driving locomotion in animals (Cohen and Wallén, 1980) provided a different solution. If newborn stepping was not reflex-driven, might more complex sub-cortical pattern generators be responsible for neonatal stepping in humans?

2.4.2 Central pattern generators – a theory

2.4.2.1 Definition of the CPG in animals
Extensive research in animals has founded the current knowledge of central pattern generators (CPGs). According to this body of work, CPGs are rhythm generating networks of interneurons within the spinal cord, which do not require - but can be influenced by - sensory input from the limbs (Dietz, 2003). CPGs are hard-wired and available from birth (Yang et al., 1998a), and are thought produce locomotion through genetically inherited (Grillner and Wallén, 1985) complex patterns of muscle contraction. They provide a readily available, simple mechanism that frees up cortical capacity (Forssberg, 1985). Evidence for CPGs were shown in studies with primitive fish, where in vitro studies showed that regions of neurons caused rhythmical movement that could produce locomotion in the lamprey (Cohen and Wallén, 1980). Research in mammals showed that cats with a spinal transections made alternated limb movements while supported on a treadmill (Grillner, 1973). Further, these steps matched belt speed (Forssberg et al., 1980, Miller et al., 1975), suggesting that adaptive locomotion can occur in cats without input from the brain.

2.4.2.2 Does a functional CPG exist in humans?
However, it was more difficult to demonstrate the existence of functional CPGs for locomotion in humans (Dietz, 2003, Yang and Gorassini, 2006, Duysens and Van de Crommert, 1998). Only indirect evidence was found in humans with spinal cord transsections (Yang and Gorassini, 2006). In one isolated case – a patient with an incomplete spinal transsection and a limited ability to walk – involuntary stepping could be elicited by extension of the hip (Calancie et al., 1994). Although this patient’s involuntary rhythmical muscle contractions resembled output from studies on involuntary animal stepping, the authors could not entirely dismiss the role of limited supraspinal tonic facilitation in the automated response. Other evidence was sought in the
resemblance of load responses – e.g., the swing initiation in the support leg after unloading at the end of stance phase - between intact humans and mammals, as in mammals this response is thought to be modulated in spinal locomotor circuits (Pearson et al., 1992). However, sensitivity to proprioceptive cues may also occur without the existence of pattern generating centres.

Clearly, the evidence for CPGs in humans is not strong, particularly as in these studies, afferent input could have been the cause of motion. The ultimate test of the existence of CPGs in humans would be to induce ‘fictive locomotion’ (Duysens and Van de Crommert, 1998). Fictive locomotion pertains to the electrical stimulation of locations in the spinal cord or brain stem in spinalised, decerebrate or otherwise paralysed animals, to elicit automated locomotive cycles (for example, alternating tail muscle bursts in fish, or flexor-extensor activity in cats). Ideally, fictive locomotion is measured through nerve root activity to exclude any motion artefact (Engert, 2013), but often, overt movement is measured. Recently, progress has been made in the realm of epidural electrical stimulation in patients with motor-complete spinal cord transsections (Danner et al., 2015). Continuous electrical stimulation of the spinal cord elicited coordinated rhythmical EMG in four muscles in supine subjects. However, even in these experiments of fictive locomotion, subjects did not remain motionless after the initiation of the stimulation and further facilitation from reflexes and sensory input from these supine steps cannot be excluded. Additionally, the completeness of the lesion is not controllable in humans, and it is not clear if surviving corticospinal connections may have contributed to the movements. A recent review emphasised the body’s ability to utilise multisensory information to generate motor patterns as an essential factor in the recent achievements in electrical stimulation interventions for patients with spinal cord injury (Hubli and Dietz, 2013). Thus, the existence of a central pattern generator for locomotion that works without sensory input has not been confirmed in humans.

2.4.2.3 Is neonatal stepping controlled only at a spinal level?

The idea that cortical influences on movement are minimal at the start of life prompted some researchers to look for evidence of a CPG in human infants. The CNS is not yet fully myelinated (Richardson, 1982), and transcranial stimulation did not elicit strong leg movements until the fourth year of life (Müller et al., 1991), suggesting that corticospinal links are not fully functional. The strongest evidence for subcortical control of neonatal stepping is the ability to elicit steps in anencephalic infants (Peiper, 1963), although it is
not always possible to evoke stepping in these children (Funayama et al., 2011). Conversely, there is some evidence that corticospinal tracts are in fact available and functional from birth (Eyre et al., 2000). The level of involvement of these pathways in stepping has only recently been demonstrated: Barbu-Roth et al. were able to elicit stepping through optic flow alone (2009), and Kanazawa et al. (2014) found high levels of descending corticomuscular coherence in free, non-stimulated newborn leg movement, suggesting that the primary motor cortex is driving spontaneous movement even at birth. Nevertheless, researchers who believe in the existence of a functional CPG in humans consider neonatal stepping as a reflection of the behaviour of the spinal and brainstem circuitry prior to maturation of all inputs from the brain (Yang and Gorassini, 2006, p. 381). Infants seem to be an ideal subject pool for the study of the CPG to these researchers.

**2.4.2.4 Adaptability in response to tactile-proprioceptive feedback**

The general focus of these studies was on the adaptability of the stepping pattern to afferent influences. Attempts were made to repeat the cat studies in human infants – logically without any of the anatomical and physiological manipulations. A series of experiments with a motorised infant treadmill seemed to imply that the central pattern generator was ‘adaptable’ to different tactile-proprioceptive perturbations. Stepping adapted to treadmill speed and a strong relationship between treadmill speed and step cycle duration was shown, although it was not clear for which age group this relationship was calculated (Yang et al., 1998a). Adaptability was also evident for direction (Pang and Yang, 2002, Lam and Yang, 2000) and induced trips (Yang et al., 1998b, Pang and Yang, 2001, Pang et al., 2003, Lam et al., 2003). The artificial extension of the stance phase (Pang and Yang, 2000) also resulted in corrections that allowed the infant to maintain stepping in alternation. A similar response was seen in the interlimb coordination on a treadmill with a split belt: when the two legs were moved at different speeds, alternation between legs was a preferred pattern with only one leg entering the swing phase at a time (Yang et al., 2005, Thelen et al., 1987c). This was explained with the existence of a half-centre model, where the flexor half-center responsible for flexion in one leg inhibits the flexor half-center in the contralateral leg. The adaptations of the movement patterns were described as predictable and well-organised (Yang et al., 1998a).

The responses were remarkably similar to those found in the cat studies, which led to the interpretation that a highly functional and adaptable CPG was present in human infants.
However, a shortcoming of the evidence described above was the lack of distinction between age groups and reporting of inter and intra-individual variability. These authors aggregated data from infants across the first year of life (for example, from 10 days to 10 months (Yang et al., 1998a) or from five to 12 months (Pang and Yang, 2000) and averaged cross-sectional data. Not only does this make it unclear how the relationships and responses change with increasing age, but it also masks individual responses. There is no detailed description of the newborn period, which, in CPG theory, should be associated with behaviour resembling the animal studies most strongly and give the most predictable responses, as cortical influences are supposedly absent. It is also possible that sensory adaptation takes place without the presence of a CPG, and the research does not directly infer that stepping originates from pattern generation. It does offer strong evidence for the role of tactile-proprioceptive input in stepping in the first year of life.

2.4.2.5 Functionality of muscle activity patterns

To illustrate the sensory adaptation on a neuromuscular level, EMG data was gathered alongside the kinematic descriptions in these studies (with infants aged between three and 12 months). Evidence for neuromuscular adaptation was found when hip position was manipulated (Yang et al., 1998b, Pang and Yang, 2000): hip flexor timing was linked to the level of extension of the hip. Load caused an increase in extensor contraction strength and duration, effectively prolonging the stance. Note that these are functional cues in walking, as high loads naturally occur during the middle of stance, while loads decrease towards the end of stance (Pang and Yang, 2000, Pang and Yang, 2002). Thus, they proposed that CPGs are flexible and show functional responses to sensory cues. Further evidence of the functionality of the CPG was given in the finding of reciprocal activation of antagonistic muscles (Yang et al., 1998a), but again, this finding was not substantiated with a quantitative analysis. One other group observed reciprocal activation of antagonists (Okamoto and Okamoto, 2001, Okamoto et al., 2003), but also quantified it poorly. In the interest of efficiency, muscles should contract reciprocally, as co-contracting antagonists counteract each other’s force. However, coactivity can also be found during the adult cycle (Di Nardo et al., 2015, Park et al., 1999), when it is used to stiffen the leg at critical times. Measures of reciprocal or simultaneous antagonist activity can only be presented as evidence if it is quantified and compared in relevant contexts. In a better quantified study, coactivity in infant stepping was found to be considerably higher than in adult walking (Teulier et al., 2012). Overall, the interpretation of reciprocal
activation and sensory adaptability as evidence for an efficient and well-developed locomotor CPG at birth seems to be poorly substantiated, particularly because none of the research focused on newborns exclusively. Furthermore, the finding that the CPG is adaptable to sensory cues does not offer evidence for the pattern generator itself. It is also important to note that these studies aggregated cycle EMG into ensemble averages (Yang et al., 1998a, Pang and Yang, 2002). These averages show generic responses without offering ‘the ability to appreciate potential plasticity in neuromuscular activation from one step to another’ (Teulier et al., 2015, p. 450). Responses to sensory cues may have been quite variable across cycles and individuals, showing that muscle contraction during stepping may be modulated by many more factors than a hard-wired central pattern generator that is sensitive to load and hip extension (Thelen and Ulrich, 1991).

2.4.2.6 The development of muscular activation patterns

As mentioned before, the study of the locomotor CPG did not thoroughly interpret how the CPG develops from the newborn period to independent walking. Although data were accumulated for a great range of ages, these were often collapsed for one experiment. For example, the occurrence of reciprocal activity was not associated with any age in Yang et al. (1998a). However, Okamoto found a relationship between developmental stages and reciprocal muscle activity in a study on one infant from one week after birth to seven years old (2003) and a study on 10 infants from one week to four months after birth (Okamoto and Okamoto, 2001). They proposed that improving balance control caused these neuromuscular changes, although they attributed the improvement of balance to neuromaturation. Most studies, however, examined infants up to 10 (Yang et al., 1998a), 12 (Pang and Yang, 2001) and 13 months (Pang and Yang, 2002) without showing the development of muscle patterns across this time. As infants have largely gained voluntary control of their movement apparatus when they are a year old, it is unlikely that a central pattern generator affects the control of stepping the same way across the whole subject group.

What role does the CPG play in the development of walking? Forssberg (1985) suggested that the innate neural networks causing infant stepping were retained in adulthood, but that they were ‘transformed to an efficient bipedal pattern by a central system that is unique for man’ (p. 481). Dominici et al. (2011), as the only study focusing on newborns in detail, found evidence for a limited number of basic neuromotor patterns that are retained from neonatal stepping to walking in toddlers, pre-schoolers and adults. In
development, these locomotor primitives were overlaid with more complex patterns, reflecting increased supraspinal, intraspinal, and tactile-proprioceptive sensory integration (Dominici et al., 2011). These authors attempted to define variability by ensemble-averaging step cycles from three groups based on cycle length and correlating them. They found that EMG was ‘similar relative to a cycle regardless of its period’ (Dominici et al., 2011, p. 997), however, they did not include measures of between-cycle variability. Additionally, most of the studies offered little specific focus on stepping development, as they did not link kinematic and neuromuscular transitions within the period of infancy.

2.4.2.7 Summary
The study of the functionality of the CPG in human infants is largely based on the assumption that CPG activity drives locomotor patterns in infants the same way as is does in reduced animal preparations. Infant stepping research primarily focuses on the ability of this functional CPG to adapt to sensory cues. CPGs for locomotion are proposed to be innate and therefore readily available at birth, follow fixed but functionally adaptable coordinative rules through the influence of connected half-centres (Yang et al., 1998a), and neuromuscular patterns are basic and reproducible (Dominici et al., 2011). However, although many areas of adaptability are shown, the existence and functionality of the CPG in humans, and particularly in infants, was not directly demonstrated. It is possible that adaptability and indeed, stepping itself, could have alternative, or more complex origins.

2.4.3 Dynamical systems view of stepping
The role of CPGs in neonatal stepping was disputed by a number of other researchers. Fundamental differences in data collection and analysis - which focused on individual responses rather than common patterns - yielded high levels of inter and intra-individual variability in kinematic and neuromuscular variables. A different approach emerged, which highlighted the existence of variability and divergent motor solutions. Furthermore, variability was given theoretical significance. With elements from Bernstein’s theory of motor control (1967), Gibson’s ecological psychology of visual perception (Gibson, 2014, Gibson, 1979) and Kelso’s application of the dynamical systems theory (Thelen et al., 1987a), Esther Thelen and associated investigators formed a more global and flexible approach (see Thelen’s review in 1995: ‘A new synthesis’).
2.4.3.1 What is dynamical systems theory?

For the purpose of clarity, dynamical systems theory and some of its key principles require explanation. Dynamical systems theory is an area in mathematic science, where it is used to describe pattern formation in nature and physics. J.A. Scott Kelso applied aspects of this theory to human behaviour (Kelso, 1995), and Esther Thelen used it to explain for developmental change (Thelen et al., 1991, Thelen, 1995). According to dynamical systems theory, patterns emerge through self-organisation. Self-organisation proceeds from ‘the relations among simple parts and without the need for prior programs or blueprints’ (Thelen, 1992, p. 189). A simple example can be given in the behaviour of water drops dripping from a tap (Thelen and Ulrich, 1991). The rhythm of the drops does not depend on a pre-set timing system, but emerges through the interplay of the coherence of the water molecules, the flow rate, the history of previous drops and other factors. Thus, the behaviour of simple parts depends on multiple factors, and changes in one, or a combination of them, may have non-linear effects. Some states can be quite stable - in other words, resistant to change - or may be unstable and ‘spontaneously shift into different patterns’ (Thelen, 1992, p. 190). These shifts are generally preceded by instability or increased variability in the pattern.

2.4.3.2 Regulation of stepping

The neuromotor patterns in stepping can be explained accordingly. The principle of multi-causality opposes the idea that stepping is principally caused by a central pattern generator. Although not disputing the existence of neural networks or the ‘possibility of reflexes having ontogenetic, adaptive functions’ (p. 773), Thelen suggested that there has been too much emphasis on central processes in the mechanism of stepping (Thelen and Fisher, 1982). Instead, she highlighted that anthropometric factors can affect the expression of stepping (Thelen et al., 1982). Firstly, Thelen observed the kinematic and neuromuscular similarities between stepping and supine kicking and proposed they were forms of the same behaviour in different postures. Contrary to stepping, kicking does not disappear, and she suggested that it was the increasing weight of the legs caused by rapid growth that inhibited stepping after the first two months of life. Kicking behaviour continues and indeed increases, because in this position the latter part of the flexion is aided by gravity. The reduction in stepping associated with increasing leg fatness (Thelen et al., 1984) may be countered by a change of context. A subsequent study confirmed this suggestion; infants were suspended upright in water, or had weights attached to their legs.
Infants in the reduced-weight environment stepped, even if they were unable to step when supported upright on a surface. In opposition, infants with increased weight on their legs reduced the number of leg movements. The disappearance of stepping appeared to be a function of anthropometric changes.

What, then, drives stepping? Fundamental is the idea that movement does not involve the recruiting of independent musculature, but emerges from functional synergies that span different muscles and joints, and takes into account anthropometric properties such as mass and stiffness (Thelen et al., 1987b) and external constraints such as environment and task (Newell, 1986). This reduces the degrees of freedom, lowering the demand on the brain through the diminishing of control decisions. Thelen proposed therefore that stepping is not driven by any particular structure or scheme, but emerges from ‘soft-assembled’ behavioural attractors (Thelen, 1992). These behavioural attractors exist as multiple components that interact with each other (see Figure 1).

![Figure 1: Hypothetical components of motor development, plotted as a function of age (from Thelen, 1986a)](image)

Considering these ideas, it is not strange that stepping is mainly studied from a developmental perspective, and attempts to explain for the different expressions of the behaviour from newborn stepping to independent walking within its own context.
2.4.3.3 Inter and intra-limb coordination

Early studies looking for evidence of functional muscle synergies in stepping focused mainly on the coordination of the limbs and joints. Controlling the many degrees of freedom in stepping is a difficult task for the neonate, and one way to reduce the demand is to simplify the task to a much simpler, functional motor synergy. Within the limb, this is evident in the tight synchronisation of joints. During the newborn period (one month of age), the onset of movement and peak excursion of ankle, knee and hip joints were strongly linked (Thelen and Cooke, 1987). In other words, the joints flexed and extended at approximately the same time. With time, the ankle joint dissociated from this coordinative structure first, with hip-knee synchronicity decreasing at a much later stage, after the attainment of independent walking. Joint individuation, according to these authors, emerged with experience.

Further evidence was found in the ability of infants to step in a left-right pattern. Alternation as a preferred mode of coordination is also used to support the idea of central pattern generation, as it can also be found in animal preparations in which the spinal cord is severed. Even on a split belt treadmill where one belt was twice as fast as the other, the preferred pattern was alternation (Thelen et al., 1987c). Cats compensated in the stance phase; they prolonged leg contact on the fast belt and decreased it on the slow belt (Forssberg et al., 1980). Similar responses were seen in kicking in healthy six-week old human infants (Thelen et al., 1987b). Thelen suggested that both limbs were integrated in a coordinative structure, but that the state of one limb modified the activity of the other with the aim to keeping a base of support below the trunk. However, conversely to Yang et al. (1998b, 2005), this study emphasised that alternation was not the only form of coordination. Other motor solutions were also used, such as single steps, steps that were repeated on the same side (double steps), and steps with both legs at the same time (parallel steps).

Thelen suggested that alternation was not a ‘fixed mode of interlimb coordination’ but a preferred motor solution (Thelen et al., 1987c, p. 408). When infant legs were not driven by the treadmill, little alternation was evident. The importance of the environment to the expression of motor coordination was earlier demonstrated in a study on hind-leg coordination in rat foetuses (Robinson, 2005). The author trained the animals in yoked hindlimb paradigms, demonstrating that alternating legs can be constrained into synchrony. Interestingly, a study with 10 day old spinalised rabbits showed that hindlimb coordination could be trained even without input from the brain. In normal situations,
rabbits alternate the hind-limbs in infancy but start hopping as rapid lengthening of the hind-limbs makes this coordination more efficient. Maturing rabbits maintained alternation when their spinal cord was cut, but, surprisingly, they were able to hop when they were trained to use their legs simultaneously (Viala et al., 1986, Viala, 2006). In natural circumstances, infants utilise all coordinative forms in stepping in their first months of life, but under the influence of experience, alternation becomes the most preferred coordination (Thelen and Ulrich, 1991). Therefore, coordination more likely depends on ‘structural constraints of the leg and the biodynamic demands of the task’ than on a CPG (Thelen and Cooke, 1987, p. 392).

2.4.3.4 Muscle activity
Thelen and her colleagues also studied coordination on a deeper, neuromuscular level. Smooth and accurate activation of muscles is generally not found in young infants, resulting in jerky, uncoordinated movement. Even for skilled motor behaviour muscle patterns are highly variable, and it is difficult to find common patterns, particularly for infants. Yet, Thelen found some recurring features in stepping. Infants tended use hip flexion to furnish the primary drive for the swing phase of the step, causing a passive flexion of the knee. The stance phase of the step was associated with relaxation rather than active extension (Thelen and Fisher, 1982). In newborns, the Tibialis Anterior (ankle flexor) was often continuously active. The Rectus Femoris (hip flexor and knee extensor), was extremely variable and showed little evidence of developmental change over time. Further, the posterior leg muscles Gastrocnemius and Biceps Femoris only showed small and sporadic activity, but this improved later in infancy (Thelen and Cooke, 1987). In summary, most muscle patterns were characterised by tonic activity and co-contraction, and Thelen did not observe clear muscular activation patterns such as those found in other studies (Yang et al., 1998a).

2.4.3.5 Coactivity between antagonist musculature
Coactivity is generally observed in young infants (Forssberg, 1985, Teulier et al., 2012, Thelen and Cooke, 1987), although Yang also found reciprocal activation (Yang et al., 1998a). As described in the previous section, this may simply be due to the method in which the data were studied. Pooling data within subjects or even across subjects and age groups to generate an ensemble average tends to mask variability, and it was not clear which age groups the analysis targeted. Systems theories explain coactivity as a
functional motor solution aimed at simplifying the task (Spencer and Thelen, 2000). The uncoupling of muscle action associated with advancing development may be caused by a decrease of general muscle tonicity and increased control. Okamoto described an increase in reciprocal contractions around the third and fourth month of age (Okamoto and Okamoto, 2001). Teulier (2012) also saw a decrease in coactivity through the first year, although it had not completely disappeared at 12 months. As co-contraction has additional functional benefits - it increases joint stiffness (Damiano, 1993) – its persistence fits well into the overall story of the development of walking, particularly when weight bearing and balance become a factor. Fine-tuning of muscle control will cause reduction of coactivity, because the improvement of balance and strength reduces the need for extra joint stability (Thelen and Cooke, 1987). However, it is expected that there is a continuous need for this stability - or reduction in degrees of freedom - especially when infants transition to new levels within the task (Vereijken, 2010); for example, when they transition to a gravity environment or learning to walk independently. It is therefore likely that coactivity in stepping is high directly after birth and during critical periods throughout infancy.

2.4.3.6 Variability
A separate note should be made on variability. Its presence throughout all features of neonatal and infant stepping is pervasive. Dynamical systems researchers view variability as a developmentally functional phenomenon. Normal variability offers flexibility and room for development, because if patterns need to be able to change, ‘they cannot be rigidly stable’ (Vereijken and Thelen, 1997, p. 91). With time, neuromotor patterns become less variable and more defined, although stabilisation of patterns cannot take place until enough experience is gained (Teulier et al., 2012). Variability is also associated with periods of transition (Vereijken, 2010, Adolph et al., 2003), or when a new skill is learned. Clark and Philips (1993) detected increased variability in the relationship between the thigh and the shank when infants transitioned from supported stepping to independent walking, even though this relationship had been stable in supported stepping. Consecutive experience through perception-action cycles (Gibson, 1979) allows for the system to reach a preferred state, resulting into a stable pattern (Thelen, 1995). However, a degree of variability is always evident in a pattern and shows its ability to adapt to a sometimes rapidly changing context, for example, belt speed (Thelen, 1986b, Thelen et al., 1987c)
2.4.3.7 Viewpoints in development

There are distinct differences in how Systems Theory views the role of stepping throughout development compared to more traditional views. Firstly, stepping is an early expression of locomotion, rather than an incomplete component that is later overlaid with functional behaviour. It may even be possible that upright stepping is part of a collection of generic neonatal locomotive behaviours such as neonatal crawling, air stepping and kicking, which all derive from functional, coordinated intra-uterine movement. Stepping is continuous from birth to adult walking – without being exactly the same at any point. Thelen acknowledged that it was difficult to prove continuity, because the behaviour disappears around the second month (Thelen and Cooke, 1987). She proposed that continuity was likely if 1) training studies proved effective, and 2) if intermediate forms of stepping linked neonatal stepping to independent walking. Evidence for the first point was observed in training studies which showed that daily training resulted in a retained stepping response and facilitated the onset of independent walking (André-Thomas and Autgaerden, 1966, Zelazo et al., 1972). However, Thelen suggested that these effects may have been caused by increasing strength rather than the development of stepping coordination (Thelen and Cooke, 1987). A consecutive study (Zelazo et al., 1993) showed that training in stepping did not improve the ability to sit, suggesting that training was selective of the trained motor behaviour and did not generalise beyond the trained pattern. Evidence for the second point was given in a study of stepping through the full age range between birth and independent walking. When stepping returned after the period of no stepping, the behaviour strongly resembled neonatal stepping. Even when infants started walking independently, features of the neonatal pattern were observable (Thelen and Cooke, 1987). Motor patterns in stepping are therefore likely continuous throughout development, and tend to evolve and regress with sudden shifts that reflect phase transitions of sub-systems.

2.4.3.8 The role of experience

Further, development is driven by experience, rather than the automatic maturation of the neuromuscular system. Directly after birth, stepping patterns seems to be very unstable, with highly variable inter and intra-limb coordination, reflecting the transition to a novel environment (Ulrich, 2010). As neonates gain experience in the world of gravity, stepping becomes considerably more organised and facilitates progression to new challenges (for example, increased weight bearing). The key driver behind development, according to
dynamical systems theory, is ‘exploration, or the active testing of the possible spaces where current skills and the desired tasks intersect, and the subsequent selection of those actions that match the functional needs best’ (Thelen, 1992, p. 192).

Adaptation can happen directly in response to an altered environment. For example, Jouen observed postural adaptations as a reaction to optic flow in newborns (2000). However, development appertains to more permanent changes and reflects the interplay between sensory feedback loops, growth and maturation, and perhaps motivation. These constructs are present even in utero. An example of the role of experience for development in the womb is illustrated by an experiment in rat foetuses by Robinson et al. (2006). These authors tested the amount of force needed to deform the uterine wall at one or two points, and found that more than twice the amount of force was needed to deform two points of the myometrium than if only one point was deformed. They proposed that this was the factor that shaped bilateral coordination: alternating movements were simply more efficient to achieve more limb extension. Examples of learned behaviour were also given in human foetuses: as hand-mouth contact became increasingly more frequent as foetuses gained experience of hand-to-face movement (Zoia et al., 2007, Reissland et al., 2014).

A further example of the role of perception in young infants is an experiment by Angulo-Kinzler et al. (2002), who found that infants as young as three months of age will adapt their kicking strategies in order to most efficiently move a mobile, suggesting that very young infants can learn goal-directed behaviour. Therefore, adaptation in response to altered environments may not just be temporary but also drive real change: augmented sensory contexts can be used to drive functional changes.

### 2.4.3.9 Summary

According to dynamical systems theory, stepping is not innate and driven by hard-wired patterns, but a soft-assembled behaviour that is highly variable and therefore not stereotypical. Dynamical systems theory does not dispute the existence of neural networks but does not attribute infant stepping to a CPG: ‘the task, not a specific pattern of muscle activations, is monitored by the central nervous system, and thus, the particular coupling pattern emerges to fit the task’ (Thelen et al., 1987c, p. 409). Stepping is continuous throughout development and influenced by many contextual factors, which explains for non-linear responses and variability. Experience gained through perception-action cycles, and not maturation alone, will stabilise stepping into a more efficient and
functional behaviour. Stepping will continue to show functional levels of variability to be able to adapt to new contexts and growth.

2.5 Eliciting steps in infants - facilitators

A result of the viewpoint that stepping is modifiable by experience has led to the application of infant stepping in training paradigms to improve step characteristics and bring forward the age of independent walking. The best learning opportunities appear to arise from repeated action and perception of movement: by eliciting many steps, often. Although spontaneous stepping in newborns occurs when infants are supported upright, or in the form of spontaneous kicking (Thelen and Fisher, 1982), it is thought that upright stepping is easiest elicited by a sensory stimulus. Indeed, there is a pervasive role for the sensory system in stepping, which is recognised by dynamical systems theory as well as the neural network or CPG theories. To optimise stepping, it is necessary to ascertain which form of sensory stimulation, or combination or stimuli, is most effective at eliciting steps throughout development.

At birth, the somatosensory system (proprioceptive and tactile) and the visual system are known to facilitate stepping. It is also known that arousal enhances the excitability of the nervous system in infants (Prechtl, 1974). It is important to note that there are very little studies of the earliest, newborn period, and therefore the following section will review the current knowledge of the effects of sensory stimulation and arousal on stepping during the first year of life with a view to explain what may be important directly after birth.

2.5.1 Somatosensory stimulation

2.5.1.1 Proprioceptive and mechanical effects of hip joint stretch

In animal models, the initiation of the swing is evoked by stretch of the hip flexor and unloading of the ankle extensor at the end of stance (Grillner and Rossignol, 1978, Pearson and Duysens, 1976). Thelen further illustrated this with an experiment with seven-month-old infants who no longer stepped on a stationary surface (1986b). When exposed to the treadmill, these infants showed improved stepping rates and coordination. Instead of march-like steps with highly synchronised joint flexion and extensions, infants produced more adult-like steps, with knee flexion initiating the swing when the hip was at maximal extension. Left-right alternation also increased. Thelen proposed that the treadmill provided a mechanical stretch that infants are not able to produce for themselves.
at this age (Thelen and Cooke, 1987) due to relative muscular weakness and poor balance on one leg. Additionally, the treadmill automatically produced a forward swing effect by bringing the leg into a position from where it can swing forward. Thus, the treadmill affords infants to utilise the ‘viscoelastic and inertial properties of the stretched stance leg to initiate swing’ (Thelen and Cooke, 1987, p. 391). Ulrich et al. (1998) elaborated on this idea in another treadmill study with infants with Down syndrome (mean age 13.3 months) and added Velcro to the infants’ socks as they walked on a carpeted belt. This was thought to enhance the ‘the interplay of the extensor muscle forces produced by the infant in stance and the translational forces produced by the moving belt’ (p. 332). In other words, they attempted to enhance movement information from the treadmill. In response, infants stepped more, showed more advanced coordination and higher vertical displacement. Ulrich noted that these high leg trajectories may have been a result of the infants’ desire to pull their foot away from being stuck to the treadmill. A study with infants with spina bifida (aged two to 10 months) did not find enhancing effects from the Velcro and proposed that these infants did not have enough strength to pull the sock loose from the belt (Pantall et al., 2011). A subsequent study showed that Velcro enhanced burst length in the lower leg and increased co-contraction in a similar subject group (Pantall et al., 2012).

These studies provide support to both mechanical stretch and forward swing theories. However, there is no clear evidence that this mechanism is functional for the newborn period; there are only few studies that manipulated extension in infants before the age of two months (Yang et al., 1998a, Teulier et al., 2012, Pantall et al., 2012) and none of these studies explicitly focused on effects of hip extension. It is assumed that newborns are also sensitive to mechanical stretch and are able to utilise forward swing, but it is possible that this sensitivity does not yet match that of their older peers.

2.5.1.2 Ankle and hip load

Some evidence was found for the effect of unloading. Pantall and colleagues found that rapidly unloading the receptors in hip and ankle joints by letting the foot drop off the end of the treadmill stimulated most muscle activity in older infants with spina bifida (aged seven to 10 months), and affected infants from the younger group (two to five months of age) less strongly (2012). Pang and Yang (Yang et al., 1998a) also investigated the effects of hip angle as a sensory gating system for swing initiation and found that the hip angle at toe off was variable. However, this variability was attributed to an interaction with
amount of load on the hip. When the load was low, the swing phase was initiated earlier, at a lesser hip angle. This is possibly a rudimentary form of functional behaviour, with the aim to maintain a base of support for longer when the load is high. In air stepping, increased load reduced leg movements in three to 10-month-old infants (Musselman and Yang, 2008, Musselman and Yang, 2007). These authors proposed that infants increased extensor support in stance under high load, and decreased flexor activity in flexion when they found it difficult to overcome a load. In fact, sensitivity to load may also manifest itself as an inability to bear weight at the ages in which infants’ strength-to-weight ratio has become unfavourable (Barbu-Roth et al., 2015). Infants may perceive the load too large when they are upright on a surface, causing the legs to collapse. These findings are in keeping with Thelen’s observations (1984) that stepping increased when four-week old infants were buoyed in water, and leg movements decreased when exposed to weights. It is likely that infants are sensitive to load or leg weight from a very young age.

2.5.1.3 Tactile stimulation
The effects of sensory stimuli against the foot sole are evident from infants’ responses to being placed on a static surface: they initiate flexion-extension cycles. However, infants also step when suspended upright in the air and it has been proposed that stepping may simply be due to increased arousal in response to upright posture (Thelen et al., 1982). It is difficult to establish the influence of the sensitivity of the foot sole, especially in newborns. The parameter has not been researched deeply, and studies that included tactile manipulations always contained elements of dynamic proprioceptive factors as well. For example, it is difficult to disentangle the effects of friction (Dycem) to the foot sole from the proprioceptive feedback that a longer stance time and deeper hip stretch may have on a friction-augmented belt. Vibration of the foot sole had varied effects on infants of different ages and depending whether they were on a static or moving treadmill belt (Saavedra et al., 2012), although it consistently decreased stepping in infants with typical development from baseline.

Another example is given in a study on 13 three-month-old infants with Down syndrome by Ulrich and colleagues (1998). These authors added a ‘nubbly’ (knobbly) texture to the belt. Infants responded with increased stepping compared to a smooth treadmill belt, but showed shorter cycle durations. It was proposed that infants’ feet were tickled and they did not like the sensation, resulting in short, evasive steps. This may be related to the shortened swing durations in Saavedra et al.’s study (2012). In newborns, mimicking the
lining of the uterus may be facilitative for stepping (Teulier et al., 2013), as this population may be easily overloaded with novel experiences. Vibration has not yet been tested exclusively in newborns. It may mimic circumstances in the uterine environment (for example, local contraction of the uterus or digestive processes) and stimulate stepping in newborns, or alternatively, cause an avoidance reaction if the stimulus is too strong.

2.5.1.4 The treadmill
A convenient way to administer stretch to the hip and unloading to the ankle, and also tactile stimulation to the foot sole, is found in the use of the treadmill. Additionally, it facilitates the capturing of data, because the subject can remain stationary. Many studies on infant stepping have used the treadmill (Thelen, 1986b, Thelen et al., 1987c, Thelen and Ulrich, 1991, Vereijken and Thelen, 1997, Ulrich et al., 1998, Lam and Yang, 2000, Lam et al., 2003, Pang and Yang, 2000, Pang and Yang, 2001, Pang and Yang, 2002, Pang et al., 2003, Yang et al., 1998a, Yang et al., 1998b, Yang et al., 2005, Pantall et al., 2011, Pantall et al., 2012, Teulier et al., 2009, Teulier et al., 2012, Moerchen and Saeed, 2012, Ulrich et al., 1995).

A further advantage is that the speed of the treadmill can be altered easily. The speeds used for infants ranged between very slow and relatively fast speeds: for example, speeds used for infants who could not yet walk independently ranged between 0.068 and 0.22 m • sec⁻¹ in a study by Teulier et al. (2009), who tested babies with typical development and spina bifida with three month intervals between one and 12 months of age, and between 0.1 and 0.6 m • sec⁻¹ in a study by Lam and Yang (2000), who tested infants with typical development between the ages of two and 12 months. Yang et al. chose speeds between 0.04 and 1.69 m • sec⁻¹ (2005) to manipulate between-belt speed ratios in a split belt experiment. The speeds in most of the experiments were between 0.1 and 0.3 m • sec⁻¹. There are no experiments that explicitly note optimal speeds for each age group, and to date, no exclusive studies are available of newborns stepping on a treadmill younger than one month. Therefore, it is difficult to extrapolate which speed is optimal for eliciting steps in infants just after birth - particularly because this may differ considerably between individuals - and a linear relationship between treadmill speed, step rate and interlimb coordination only tends to develop after the first four months in most infants (Thelen and Ulrich, 1991). Yang et al. (1998a) reported that stepping became less consistent at the higher and lower ranges, and although they did not explicitly report the range of speeds
utilised, a scatterplot across a sub-sample of nine infants seemed to indicate that younger infants between one and four months stepped optimally at lower speeds (between approximately 0.1 and 0.25 \text{ m \cdot sec}^{-1}), while older infants between four and 10 months made most steps at higher speeds (between approximately 0.3 and 0.45 \text{ m \cdot sec}^{-1}). Teulier \textit{et al.} (2012) found that a somewhat lower speed (0.144 \text{ m \cdot sec}^{-1}) was the optimal treadmill speed across the first year of life. Based on these values, a range between 0.1 and 0.25 \text{ m \cdot sec}^{-1} may be suitable for treadmill stepping in typically developing newborns, although it is possible stepping may also be elicited at even lower speeds.

\textbf{2.5.1.5 Concerns in treadmill use}

Although the treadmill offers a wealth of different options - for example, texture changes (Ulrich \textit{et al.}, 1998, Pantall \textit{et al.}, 2011, Pantall \textit{et al.}, 2012), the adding of visual augmentation (Moerchen and Saeed, 2012, Pantall \textit{et al.}, 2012) and directional differences (Thelen \textit{et al.}, 1987c, Yang \textit{et al.}, 2005) -, it is important to note that there are issues in the use of treadmills in adults. The kinematics of treadmill walking is reasonably similar to overground walking (Riley \textit{et al.}, 2007), although peak breaking force and medial shear forces were smaller on the treadmill. Other studies have found differences in joint range of motion, stride length and cadence when running (Pearce \textit{et al.}, 1983), and walking (Alton \textit{et al.}, 1998) on the treadmill. Dingwell \textit{et al.} (2001) suggested that differences caused by changing locomotor control might be masked if they are elicited on the treadmill. These differences may also be reflected in infants. Ulrich \textit{et al.} (1996) compared treadmill stepping with supported walking in newly walking infants, and found that the treadmill elicited more stable swing and stance durations; fewer muscle bursts and less inter-individual differences; less antagonist coactivity at touchdown; and more neuromuscular organisation during stance. Thelen suggested that the treadmill shifts infant stepping behaviour forward on the developmental timeline (1986b). However, infant stepping is not the same as adult walking; the differences seen between treadmill walking and overground walking likely reflect adaptation to changing balance and propulsion requirements, in adults and new walkers alike. Infants do not need to control these factors in supported stepping and therefore the differences between treadmill stepping and supported stepping are probably smaller than the differences between treadmill walking and overground walking in adults.

This highlights another consideration when the treadmill is used for training. Subtle factors present in overground walking are removed when stepping in a controlled
treadmill environment. An example of this is the absence of experience at object negotiation or dynamic balance in treadmill training. Additionally, propulsion is not used during supported treadmill stepping, but because in natural development, this skill is only integrated later during independent walking (Adolph et al., 2003, Bril and Brenière, 1993), it may not be essential to provide environments that support the development of propulsion. Additionally, the treadmill does not offer the peripheral and central optic flow that is normally experienced when moving through the environment. This might be a more important factor (Teulier et al., 2013) and is further described in paragraph 2.5.2.

It should also be noted that the effectiveness of treadmill training for infants with Down syndrome did not directly translate to other at-risk groups. Preterm infants (Angulo-Barroso et al., 2013) showed improved stepping quality but not an earlier walking onset. There is not much support for the use of treadmills in infants with cerebral palsy (Smith and Bompiani, 2013) although evidence exists for older children (Damiano and DeJong, 2009). Additionally, some case studies showed the potential of treadmill training in an infant with intra ventricular haemorrhage (Bodkin et al., 2003) and a toddler with spinal cord injury (Heathcock et al., 2014). It is not clear why these differences occurred. The interaction between the infant's stepping characteristics and the environment allows for the emergence of certain behaviours in some infants but not others, depending on the deficiency. This confirms, again, the complexity of the interacting factors in the development of independent walking (Teulier et al., 2015).

2.5.2 Visual stimulation of stepping

Although functional on many aspects, the visual system is relatively inefficient in newborns (Bronson, 1974, Del Giudice, 2011). Considering that foetuses respond with brain activity to visual stimuli when they are still inside the womb (Eswaran et al., 2002, Eswaran et al., 2004, Fulford et al., 2003), it is expected that newborns have some capacity for vision. Neonates are sensitive to patterns (Fantz, 1963) contrast (Adams and Maurer, 1984) and motion (Braddick et al., 2003, Aslin and Shea, 1990), but have little control over intraocular musculature and are slightly hyperopic (Cook et al., 2003). Because infants have limited visual experience in the womb, it is possible that the visual system is structurally ready but needs considerable experience to optimise and further develop the neural pathways used in visual perception through axonal myelination synaptic pruning (Joseph, 2000). Similarly, before birth there was little opportunity to utilise visuo-motor pathways, and therefore, the link between vision and motion may still
be primitive. Gilmore et al. (2007) showed that motion-processing systems undergo extensive development in early postnatal life. However, visual control is essential for adult locomotion (Lee and Kalmus, 1980) and there are evolutional benefits for biological preparedness for locomotion at birth (Teulier et al., 2013).

2.5.2.1 The visuo-motor link
Functional links between vision and the motor system have been found in newborns. Jouen et al. (2000) showed that newborn responses to a backward optic flow resemble adult responses. Adults, when exposed to optic illusions of forward motion, will describe a feeling of motion and respond with backward postural sway to compensate. Jouen measured this ‘vection’ in three-day-old infants, exposing them to a backward optic flow passing bilaterally to the infant while reclined in an infant seat. The detection of head pressure showed that infants actively leaned their heads back when exposed to the optic flow. More importantly, there was a relationship between head pressure and the velocity of the optic flow: the faster the flow, the more backward head pressure they exerted. Although Jouen and colleagues did not attribute neonatal sensitivity to optic flow to direct corticospinal pathways, it was suggested that ‘indirect neural tracts’ (Jouen et al., 2000, p. 208) were responsible for the adaptation of muscular activity. Van der Kamp and Savelsbergh offered a different solution and suggested that ‘vision for action and vision for perception are dissociated from birth’ (2000, p. 240). Vision for action may be more advanced than vision for perception in newborns. However rudimentary, it is clear that the visual-motor pathway is functional at birth.

2.5.2.2 Vision and locomotion
Additional to the visual-postural link, there appears to be a relationship between vision and locomotion. Barbu-Roth et al. suspended three-day-old babies over a table with different visual stimuli projected on it, including an optic flow simulating forward motion. Air stepping improved under the influence of the forward optic flow condition compared to rotating optic flow or a static view of contrasting shapes (Barbu-Roth et al., 2009). Further investigation showed that the number of air steps elicited by optic flow was similar to the number of steps elicited by contact with a solid surface (Barbu-Roth et al., 2013). Direction did not significantly influence the number of steps, but the kinematics of the knee joint were slightly altered when a backwards flow was shown.
These studies suggest that newborn stepping is sensitive to optic flow, and that optic flow seems to be equally able to evoke stepping as tactile stimulation in newborns. However, the link between vision and stepping might be weaker in newborns than in older infants. In infants between five and 10 months of age, a chequered pattern imprinted on a treadmill belt improved stepping significantly above the control condition of a solid black or solid white belt (Moerchen and Saeed, 2012). However, Pantall et al. (2012) saw that although infants between two and five months responded to optic flow with an increased likelihood of muscle activity, older infants (between seven and 10 months) responded more strongly to visual stimulation. These studies suggest that the rudimentary relationship develops as a function of experience or age. This is illustrated by the strengthening of the visual-motor link as infants gain experience of movement (Higgins et al., 1996). It might be more difficult to observe changes as a function of optic flow during the newborn period.

Interestingly, it is not only the stimulating effect of optic flow on stepping that is important in the development of independent walking. Anderson et al. (2001) describe a strong reciprocal relationship between optic flow and locomotion. Self-locomotion is associated with increasing sensitivity to optic flow direction (Gilmore et al., 2004, Gilmore and Rettke, 2003) and an improving ability to selectively respond to stimulation of portions of the visual field (Higgins et al., 1996), suggesting that the visual system develops as a function of locomotion. Self-produced movement introduces a new framework of perceptual input which allows infants to associate forward movement with radial (expanding) optic flow in the central field of vision and lamellar (linear) optic flow in the periphery - particularly because infants tend to look in the direction of movement. Repeated interpretation of optic flow sensitises the visual system to radial and lamellar optic flows, which supports the development of the postural control needed to sit, stand and walk independently (Anderson et al., 2001, Higgins et al., 1996). Thus, vision develops through experience of locomotion, whilst locomotion develops in response to improving visuo-motor experience.

The interplay between vision and walking is complex and is evident early in life. This suggests that stepping might be controlled at different levels in addition to specific centres in the spinal cord and brain stem (Teulier et al., 2013). The knowledge that optic flow facilitates stepping may be useful in clinical settings or for enhancing the sensory experience in treadmill training paradigms. Additionally, appropriate visual stimulation may facilitate 'training' of the visual-motor link.
2.5.2.3 Optic flow

When exposing newborns to optic flow, it is essential to regulate certain factors with care. The amount of light requires optimisation so that the newborns can see the flow, without being blinded. Bright lights can irritate the newborn visual system or cause retinopathy in preterm infants (Glass et al., 1985). Luminance levels used in infant vision studies ranged from 31.2 cd/m² for newborns (Gardner and Karmel, 1984) to a mean luminance of 80 cd/m² for infants between six and eight weeks (Aslin and Shea, 1990).

High contrast is preferred for neonates; the majority of studies use black and white stripes or dots on black surfaces or screens. When exposing infants to motion simulating optic flow, it is important to reduce rhythmical local luminance changes, as infants are sensitive to flicker (Aslin and Shea, 1990) and may respond to its rhythmicity rather than the direction or speed. The location of presentation of optic flow varied for different experiments. Jouen (2000) showed that the peripheral field of view is already sensitive to optic flow at birth, and Barbu-Roth was successful at eliciting steps while presenting the flow in a contextual location: the surface underneath the infant (2009, 2013, 2015). At birth, there is no preferential location for optic flow; the difference in the effects of peripheral and global optic flow on posture is first seen in infants who have experience of self-locomotion (Higgins et al., 1996). It is thought that the sensitivity of the field of view (central versus peripheral) for visual stimuli develops as a function of visual experience (Barbu-Roth et al., 2005, Lejeune et al., 2006).

However, infants appear to have a precocious sensitivity to optic flow speed. Jouen (2000) presented optic flow to neonates at speeds between 0.01 and 0.12 m · sec⁻¹ (2.5 and 30 deg · sec⁻¹) and found increased head pressures at higher speeds. Barbu-Roth also (2013, 2015) found that neonates were responsive to fast speeds (0.17 m · sec⁻¹, or 23 deg · sec⁻¹). Infants appear most sensitive to optic flow speeds that are similar to treadmill belt speeds optimal for eliciting stepping.

2.5.2.4 Evidence for the existence of corticospinal motor pathways

The ability of the newborn motor system to respond to optic flow offers further evidence that stepping is driven by more than a spinal pattern. However, it does not explicitly show involvement of higher brain structures, as early visual ability might manifest in the brain stem first (Joseph, 2000). Nevertheless, the infrastructure for cortical control of movement seems to be functional at birth: an anatomical study showed the presence of corticospinal pathways in neonates 26 weeks post conception, and a neurophysiological
follow-up showed that these pathways were functional, at least from birth but likely also before (Eyre et al., 2000). More specifically, myelination of reticulo-spinal and vestibulo-spinal pathways commences early in life (Ten Donkelaar et al., 2014). The functionality of corticospinal pathways was questioned by Yang and Gorassini (2006) as at that time, there were no current feasible ways to test it. However, a more recent study (Kanazawa et al., 2014) found that electrical activity in the primary motor cortex and the muscles showed strong coherence during spontaneous supine leg movements (i.e., kicking). Furthermore, they showed that the direction of this coherence was mostly descending, suggesting that it is likely that spontaneous movement is already under some level of cortical control and that corticospinal pathways are functional at birth. Even if this does not directly show that corticospinal pathways are employed when newborns step under the influence of optic flow or treadmill movement, it does illustrate the possibility of early cortical involvement, and makes it less plausible that stepping is primarily driven by CPGs.

2.5.3 Arousal

2.5.3.1 What is arousal and how do we measure it?
Arousal refers to an organism's ‘overall level of functioning at any given period of time on a continuum ranging from deep sleep to awake, alert, and active’ (Brackbill, 1971, p. 17). Although arousal is not a sensory system but a physiological process, its effect on motor behaviour in infants is substantial. Accordingly, stepping is highly responsive to arousal, with increasing levels of arousal being associated with a greater step and kick frequency in newborns (Thelen et al., 1982, Thelen et al., 1984), as well as a shorter stepping onset latency (Domellöf et al., 2005).
Arousal can be measured directly with somewhat invasive methods. As these are not suitable for movement research and would put too much stress on an infant, Prechtl (1974) devised a method of categorising infant arousal through behavioural observation. Direct correlates of arousal such as heart rate, brain activity measured by EEG and respiration rate were related to visually observable parameters by Prechtl (1974). He disputed the idea of a continuous scale of arousal in infant, as categories based on discrete criteria could only be interpreted as distinct modes of behaviour. His five-category behavioural classification included ‘asleep’ as the category with the lowest arousal, and ‘crying’ as the category with the highest arousal - and several states in between.
2.5.3.2 The relationship between arousal and movement in infants

Few studies relate arousal directly to motor behaviour. It is accepted that infants must be somewhat aroused in order to be able to move. It is not entirely clear which effects arousal has on the quality of movement, although some research refers to it briefly. For non-specific spontaneous infant movement, Hadders-Algra and colleagues found an increase in rate and vigour (1993) in three to seven day old infants. This may be attributable to ‘increased motor neuron recruitment and/or increased firing rates’ (Thelen et al., 1984, p. 489). Similarly, Vecchierini-Blineau and Guihneuc (1982) found improved membrane excitability with higher arousal in children under three years of age. Although there was no significant relationship between arousal and spontaneous kicking, Thelen found that newborns who kicked and stepped more tended to be more aroused (1982). She later found an after-effect of stepping: when seven-month-old infants were supported over a stationary treadmill after stepping on a moving treadmill, they made more steps than when they had been tested before the procedure. She drew a parallel between this ‘general tonic excitation’ and the effects of arousal at birth (Thelen, 1986b, p. 1504).

There seems to be a reciprocal relationship between arousal and movement: movement causes increased arousal as well as being facilitated by it. In young infants of two months it was noticed that the act of lifting infants into the upright stepping position increased their arousal level to show some distress (Thelen et al., 1982). This raises the question of whether there is a close relation between young infants’ overall response to test procedures and the eventual outcome that is measured. Indeed, some investigators used arousal to raise the effectiveness of the testing session: in order to elicit more steps, arousal levels were increased through play (Musselman and Yang, 2007). It is therefore strange that arousal was only recorded by a limited number of researchers; Thelen’s research group controlled for arousal in their early stepping research (Thelen, 1986b, Thelen et al., 1984, Thelen and Fisher, 1982, Thelen et al., 1982), Domellöf, Rönqvist, & Hopkins (2007) recorded arousal in their studies on stepping laterality, and Barbu-Roth and colleagues excluded infants who cried excessively (Barbu-Roth et al., 2009, Barbu-Roth et al., 2013, Barbu-Roth et al., 2015). It was not clear how many studies included excessively crying infants in other stepping studies. Interestingly, one study in which infants were crying during testing - according personal communication with the principal researcher - showed considerably more predictable responses and lower levels of variability (Dominici et al., 2011). It is possible that arousal was considerably higher in these investigations than in the studies that controlled for crying. Even from the limited
research available on the effects of arousal on movement quality, it is not inconceivable that there may be a relationship between variability and arousal in newborns. If this is true, perhaps arousal may cause changes to other kinematic and neuromuscular variables as well. It is therefore important to control for arousal and, indeed, even study it as a control variable.

2.6 Timing of treadmill training

Exposure to intervention is especially fruitful when they are timed as early as possible, when a child's neuromuscular system is at its most plastic (Blauw-Hospers and Hadders-Algra, 2005). Anderson, Barbu-Roth and Teulier (2013) highlight the importance of critical periods, during which stimulation of development is most effective. However, some of those who consider stepping a disappearing response inhibited by a maturing cortex resist the justification of training paradigms on grounds that ‘active efforts to retain these reflexes may, in fact, retard normal development or may even cause neuromuscular abnormalities’ (Thelen and Fisher, 1982, p. 761). This idea may have erroneously caused researchers to postpone training until cortical maturity was evident (Schlittler et al., 2011). Therefore, it is important to know that training supports functional neuromotor pathways for stepping that are continuous between the newborn period and later infancy. It is also essential to verify that training is not effective because it simply improves strength.

Increasing bodies of research show the importance of early and aggressive rehabilitation (Ulrich, 2010). The aforementioned window of the first two years of life is very short, especially if training only begins after the first half year has passed. Ulrich's initial training study started infants on the treadmill when they were able to sit independently for at least 30 seconds. Other training paradigms have showed effects between the ages of five and 12 months (Bodkin et al., 2003, Ulrich et al., 2008). It would be valuable to know if training can start earlier, for example, directly after birth. This would be especially useful because stepping is still easily evoked at this age. Currently, there is no research that exposes newborn infants to treadmill stimulation. It is not known if newborns will respond to, or even tolerate, stepping on the treadmill. It is also not known if training will be effective from this age.
2.7 Summary

It is clear that the ontogeny of infant stepping is still highly debated. Although neuromaturational and CPG viewpoints of stepping have become more complex and adopted some dynamical systems theory perspectives by accepting that there may be continuity between early locomotion and walking (Dominici et al., 2011), it is limited to interpretations of neuromuscular substrate. Findings from studies with a dynamical systems view have shown that stepping is affected by many internal and external factors, is exceedingly complex and variable between and within individuals, develops in non-linear trajectories, shows variability in transition periods and stabilises as a function of experience. Additionally, it places the development of stepping in a developmental continuum and links it to other infant perceptual-motor abilities: it is capable of revealing the bigger picture. Importantly, dynamical systems theory has given rise to the use of treadmill training for infants at risk of developmental delay and, because of its recognition of the importance of experience and sensory feedback, continues to generate valuable research. Studies focused on the role of tactile-proprioceptive stimulation by varying surfaces (Ulrich et al., 1998; Pantall et al., 2011; 2012), modifying speed of the treadmill stimulation (Thelen, 1986) and stimulating the legs separately, with different speeds (Thelen et al., 1987). Findings suggest that the treadmill advances stepping, including in groups in which stepping is not easily elicited, and moreover, improves the quality of movement behaviour (Thelen 1986). Others have investigated the role of optic flow, either in combination with or without treadmill stimulation, and found that there is a rudimentary link between stepping and locomotion from birth (Barbu-Roth et al., 2009), which strengthens as infants gain visual experience while moving. There appears to be evidence for the presence of functional corticospinal pathways at birth (Kanazawa et al., 2014), although these are not necessarily responsible for the visuo-motor link. Additional to external stimuli, arousal strongly affects movement behaviour at birth, and it is essential to study arousal alongside movement behaviour. Finally, a knowledge gap exists for newborns, justifying a detailed investigation of sensory stimulation sensitivity in this age group.
Chapter 3:
METHODOLOGY
3.1 Introduction

Because this thesis presents the findings in the form of journal articles, a brief methodology is described in each chapter. However, the main methodological considerations are described in this methods section. This includes issues in recruitment and data capture common for infant research, coding details, considerations in EMG methodology and the specifics of the statistical analyses performed in this thesis.

3.2 Ethics

Newborns were recruited from the Port Royal maternity hospital in Paris (28 in Experiment 1 and 24 in Experiment 2). Infants were selected for recruitment if they were healthy; had an Apgar score of 10 at the 5th minute; were of a minimum birth weight of 2500 grams; had a gestational age of minimally 38 weeks; and had uncomplicated delivery. Parents who were interested to partake were invited to call the movement laboratory by phone when the infant was feeding so that testing could take place directly after the newborn had been fed. Newborns and at least one parent were then escorted to the laboratory.

In Experiment 1, seven infants were excluded from the final sample - one for excessive crying before the start of the experiment, four because they fell asleep shortly after the experiment started, and two because they performed fewer than 10 steps during the testing. Twenty-one infants were therefore included in the analysis (11 male, 10 female; weight 3254.9 (±402.5) grams; age three (±1.8) days; mean term 39.5 (±1.1) weeks). In Experiment 2, four newborns were excluded from the final sample because these infants fell asleep during the experiment. The final sample included 20 newborns (12 m, 8 f; birth weight 3367 (±482.8) grams; age 2.7 (±0.7) days; mean term 40.1 (±0.9) weeks).

Ethical approval was obtained from the ethics medical board of Paris-Ile de France and parents and caregivers gave informed consent for infant participation.

3.2.1 A note on the vulnerability of newborn subjects

It is important to strike an appropriate balance between testing time and data richness in infant research. The first consideration in studies with newborns is that it takes time to recruit suitable numbers of newborns to participate. Further, it is difficult to access the subjects at the right time, because newborns are often asleep during their first weeks of life, particularly in the first 48 hours after birth. They do not have great tolerance for extended procedures, and often change behavioural state during testing. Deviation from
optimal arousal level and the need for speed makes it difficult collect perfect data, and often causes the exclusion of subjects or trials. According to Thelen (2000), it requires ‘a great deal of skill and patience’ to use sophisticated techniques in young subjects (p. 391). Particularly when collecting electromyography (EMG), large amounts of extra data may have to be collected to capture an appropriate sample of the preferred behaviour (Woolacott and Assaiante, 2002). Therefore, infant research is often characterised by gaps in the data or low sample size (Thelen and Cooke, 1987; for a complete overview of included data, see Appendix B). Overall, the collection and analysis in infants is complex, which translated into difficulties in statistical testing in this project.

3.2.2. Impact of testing equipment and clothing on stepping

Newborns were tested wearing their diaper and a vest. For both experiments, electrodes were also placed bilaterally on the upper and lower legs, and for Experiment 2 alone, retroreflective markers were placed on the joints (neck, shoulders, elbows, wrists, hips, knees and ankles). It is possible that external testing equipment and the diaper affected the movement patterns of the newborns. However, the electrodes and markers were very small and did not add much weight (less than 0.5 % body weight of the smallest infant), even though the sensation on the skin may have been perceived as discomfort by some newborns and consequently increased their arousal level. However, it is not thought that testing equipment affects movement rate significantly (Groenen et al., 2010).

Conversely, diapers are bulky and positioned where they can affect leg movement. They negatively affect function and proficiency in 13 and 19-month-old infants (Cole et al., 2012), which was observable in increased falling and less mature walking patterns (a wider stance, shorter step length and smaller dynamic base angles) when infants were walking with a diaper on compared to walking naked. Bulk and weight between the legs affected stance width and thus step length and balance. As balance becomes only an issue when infants start to walk independently, a diaper may not affect the functionality of newborn stepping greatly. However, it forces newborns to adopt different stepping kinematics than they were accustomed to in the womb, where their legs could move more parallel to each other. Additionally, in combination with testing equipment, diapers have been shown to reduce stepping significantly in two to four-month-old babies (Groenen et al., 2010). In the current project, the diaper may have altered the natural newborn movement pattern by reducing step range of motion along the longitudinal treadmill axis. However, step length was not the main variable tested, and even though forward potential
improvements in certain conditions or arousal levels may have been attenuated, the benefit testing the newborns naked did not outweigh the disadvantages. Nonetheless, it is possible that in this project, step rate and range of motion were reduced compared to newborns’ true ability.

3.2.3 Feasibility of newborn treadmill training
A note should be made on the achievability of treadmill training from birth. The relatively large percentage of excluded infants (25% in Experiment 1 and 17% in experiment 2), suggests that treadmill training does not elicit a response in every case in newborns with typical development. This may be even more true in infants with a disability or at risk of developmental delay, suggesting that treadmill training may not be highly feasible at birth. For example, Pantall et al. (2011) examined stepping in infants with spina bifida and noted that infants of the youngest group (two to five months of age) performed a very low amount of treadmill steps. Absolute step rate ranged from approximately between 0.07 or 0.08 steps per second to 0.2 steps per second, which appears to be quite low compared to infants with typical development, especially considering that Thelen and Fisher (1982) found an average of 17.1 steps per minute (or 0.285 steps per second) on a static surface in infants with typical development of the same age (1982). In a direct comparison between one-month-old infants with typical development and spina bifida, Teulier et al. found that typical developers made on average approximately 0.45 steps per second, whereas infants with spina bifida made approximately 0.25 steps per second on the treadmill at one month of age (2009).

However, it is important to consider that in the current project, high and low arousal was the main criterion for excluding infants from the analysis. In Experiment 1, five infants were excluded because they were too distressed or asleep (18% of sample), and only two infants were excluded because they did not produce more than 10 steps in total (7% of sample). In Experiment 2, no infants were excluded due to low response while at appropriate arousal levels (see Appendix B for an overview of excluded infants across the two experiments). The strong link between arousal and stepping on the smooth treadmill belt suggests that if the excluded, under-aroused newborns had been at the appropriate arousal level during testing, steps would have been made. In training settings, the treadmill would be readily available to parents, so that training can take place when infants are at appropriate arousal levels. Therefore, basing feasibility on the number of all excluded infants would not be correct. In fact, across 52 infants, only two alert infants
(4%) did not respond to the treadmill with steps. It is important to acknowledge that there will always be infants who respond less to treadmill stimulation, which is the case for typically developing infants (with step rates ranging between approximately 0.1 and 0.75 steps per second) and infants with spina bifida alike (ranging between 0 and 0.75 steps per second). However, it is important to understand that all infants have an individual developmental timeline, and even in incidences of low response, development may still be modified in subtle ways. And in turn, response rate may increase as infants gain critical abilities with training.

3.3 Coding

3.3.1 The construction of a coding scheme
Because none of the current coding schemes have focused on behaviour of newborns on the treadmill, the construction of an entirely new coding guideline was deemed necessary. Eleven sagittal-view videos from E13 were imported into Siliconcoach software (Siliconcoach Ltd, Dunedin, the New Zealand, version 7-0-2-2) and globally assessed by two coders. Five distinct categories were found. In the first category (glide), the infant slid the foot forward along the belt, without lifting off the belt surface. The second category (kick) consisted of a simple hip flexion with forward or rearward excursion of the leg, with minimal knee flexion. The third category (foot play) contained various foot movements, for example, curling the toes on the treadmill surface. The fourth category (pump) showed an up-and-down pumping action of the hip and knee in a vertical plane. Lastly, the fifth category (step) consisted a typical, clear stepping movement with a swing and a stance phase.

Following this categorisation, a reliability assessment was performed by two coders on 87 movements to verify if the coders agreed on the movement they observed. It became clear that the categorisation was too detailed: most movements fell into either the step or the pump category, and the differences between the smaller categories were not often clear. It was decided to focus on the two main, most step-like movements. Distinct between these movements was the level of forward potential in the upright position: steps could support forward translation whereas pumps principally showed vertical leg movement. Upon reassessment of the other movements, ‘kicks’ and ‘slides’ fit into either the step or pump category. If they did not, they did not resemble stepping enough to be included in the analysis.
Before finalising the coding scheme, reliability analyses were performed over six trials from randomly selected subjects, looking at 12 sub-classifications. These sub-classifications contained time stamps (start, touchdown and end of movement, with a positive and negative error margin of 0.1 second), laterality, backwards motion, movement type, sequence type, double touch-down, step size, the position and location of touchdown, and the foot position during stance. Coding guidelines were adjusted until the criteria were clear and repeatable for both coders.

3.3.2 Movement coding

All videos were coded frame by frame in SiliconCoach. Recording of the movement commenced when the foot moved forward for more than 0.5 seconds, after which the coding flow chart (Appendix A) was followed to record the timing and classification of the movement. More detailed criteria are described in the coding guidelines (Appendix A). Details were entered in a Microsoft Access database.

3.3.2.1 Movement type

The two movement types that were recorded, steps and pumps, could be distinguished by a difference in forward potential. Pumps, characterised by flexion-extension in the vertical direction, would not allow for forward movement in the upright position if infants were able to balance themselves and bear their own weight. This movement resembled marching in place in mature walkers (see Figure 2a). The swing phase of the pump typically showed little range of movement, with the foot travelling forward less than half the length of the infant’s own foot. Alternatively, the leg remained flexed for more than 3 seconds. The latter part of the movement showed a brief touchdown followed by a very short (less than half a foot-length) stance phase or an immediate subsequent flexion. A step, in contrast, showed more features of forward walking, with larger range of movement in the swing and stance phase. The foot needed to touch down at least half a foot-length forward of the starting position, with a clear stance phase following in sequence. For steps, the stance phase measured longer than half a foot-length.

3.3.2.2 Step quality

Steps were further classified by quality, with the clearest, largest steps that resembled adult walking most strongly categorised as typical steps. For these steps, the foot touched down ahead of the centre of the hip joint during the swing phase (see Figure 2c, in which
the foot touched down behind the hip). A clear, uninterrupted stance phase longer than a foot-length followed the swing phase (Figure 2b). In comparison, poorer steps that were smaller and less smooth were marked as atypical steps. Criteria for these steps were 1) absence of a clear forward swing resulting in a touch-down behind the centre of the hip; or 2) shorter stance phases (less than a foot-length); or 3) stance phases that were interrupted by brief foot-lifts (less than 0.5 s) or when the foot did not move with the treadmill belt for less than 0.5 s, or slid forward during the stance phase (Figure 2d). Note that in this instance, the forward foot motion did not mark the initiation of a new step but resembled a mere interruption within the global movement when reviewed in real-time.

Figure 2: Visualisation of step classifications. The left leg (in grey) is depicted multiple times to show the progression of the movement. Figure a shows the vertical motion of pumps; Figure b depicts the range of motion (larger than one foot length) of a typical step; Figure c displays limited swing ROM and touchdown behind the hip centre as seen in the atypical step, and Figure d visualises the shuffling of the left foot in an interrupted stance phase.

3.3.2.3 Step coordination

For steps, interlimb coordination was determined, using criteria established by Thelen and Ulrich (1991). Unilateral steps with one leg were labelled as single steps, either in the form of an isolated step, or followed by a pump, or as a sequence of repeated stepping on one side (Figure 3a). When the left and the right leg stepped approximately at the same time, both steps were recorded as one parallel step. The criterion for these steps was that they were initiated between the beginning and first 20% of a step cycle on the opposite leg (Figure 3b). When both legs moved opposite to each other, the two steps were both marked as alternating steps. For alternating sequences, a step must be initiated between 20 and 80% of a step cycle on the opposite leg (Figure 3c).
Figure 3: Visualisation of coordination types with a) single steps; b) parallel steps; c) alternated steps and d) serial steps.
A new category was described by Barbu-Roth and colleagues (2013), who observed that newborns alternated some steps within a longer time frame. Similar behaviour was seen in the current project, and the serial step was adopted as a fourth category. Serial steps were initiated after 80% of a step cycle on the opposite leg and up to one second after the end of the step cycle on the opposite leg (Figure 3d).

3.3.3 Arousal coding

3.3.3.1 Coding scheme
Arousal was assessed throughout the entire trial in five-second consecutive windows. For each window, the predominant level was recorded. Frontal and lateral videos were examined for three observations: 1) if the eyes were open or closed; 2) if the infant moved and 3) if the infant vocalised or cried (see Table 1).

Table 1: Behavioural State Assessment Checklist (adapted from Piek 2006)

<table>
<thead>
<tr>
<th>State</th>
<th>Eyes open</th>
<th>Movements</th>
<th>Vocalisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>No movements at all, extremities including head completely lax</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>Small arm movements (sometimes), from the elbow</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>No major movements</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Gross leg movements only</td>
<td>None, but it is possible that an occasional vocalisation occurs (but not of a crying nature and with interruptions larger than three seconds)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gross arm movements only</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gross arm (whole arm) and or leg movements; neck and head movements, sometimes writhing.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>yes/no</td>
<td>Gross movements with all body, writhing/tonic</td>
<td>Crying/fussing, distressed vocalisations with interruptions of less than 3 seconds</td>
</tr>
<tr>
<td>6</td>
<td>yes/no</td>
<td>Gross/tonic</td>
<td>Distressed, full-blown crying, Vocalisation can be ‘higher’, and each inhalation is followed by a cry.</td>
</tr>
</tbody>
</table>
Arousal coding was used for three purposes: 1) to exclude infants who were under-aroused (state 1 and 2) from the analysis; 2) to track changes in behavioural state throughout the experiment to ensure the results were not affected by an increase in arousal in response to certain conditions or the length of the testing and 3) to assess the relationship between movement characteristics and arousal. For the movement-arousal relationship, only the time in which infants were actively moving (state 4, 5 and 6) was analysed; the distinction was made between non-crying (state 4) and crying (state 5 and 6).

3.3.4 Reliability

3.3.4.1 Reliability of the movement coding scheme

A final test of reliability was performed on the coding scheme to assess if the observations of the coder were repeatable. Percent agreement calculates the number of agreement scores divided by the total number of scores (McHugh, 2012). Because this measure does not account for agreement occurring by chance, it is advised to also perform a calculation of Cohen’s Kappa ($\kappa$). The diversity of the possible answers to the sub-categories made it impossible to calculate an overall $\kappa$. When calculating $\kappa$, each response needs to be of the same number of categories. However, in the category ‘movement type’ it was possible to choose between ‘step’ or ‘pump’, whereas in the category ‘coordination’ the choice fell between ‘single’, ‘parallel’, ‘alternated’ and ‘serial’, and in the timing categories, time stamps were either the same or not (with a margin of 0.1 seconds). McHugh (2012) poses that when the coding is performed by experienced raters, it is safe to rely on percent agreement to determine reliability. Therefore, for movement coding, percent agreement was chosen to reflect the project’s reliability.

In this project, 11% of the trials from Experiment 1 were re-coded by the original coder and 9% by a second experienced coder. First it was verified if each coder observed the same steps. Then, for each step observed by both coders, the observations for each subcategory were compared. The total agreement score was divided by the total number of comparisons, resulting in an inter-rater reliability of 86.1%, and an intra-rater reliability of 87.3%.
3.3.4.2 Reliability and validity of the arousal coding scheme

To assess the validity of the six-state arousal coding scheme used in this project, it was compared with a different approach used by Barbu-Roth et al. in 2009 and 2013. The latter method codes crying time directly by marking the time stamps of the start and finish of a crying vocalisation. The entire Experiment 1 was coded utilising both methods, and the length of crying was compared trial by trial. A strong positive relationship ($r^2 = 0.93$, $p = 0.001$) existed between the two methodologies, suggesting that the current method is effective in its assessment of high arousal during movement in newborns.

To examine the inter and intra-coder reliability, 71 trials of Experiment 1 were re-coded by a second experimenter and the entire experiment was recoded by the original coder. The state code assigned to each five-second window was compared between the two coders or, in case of intra-coder reliability, between first and second coding. The agreement criterion was met if the states were the same. Additionally, because the number of options was the same across the entire experiment (six states), it was possible to calculate a value for $\kappa$. The reliability between coders was reasonable, with a percent agreement of 70.5% ($\kappa = 0.601$). Intra-rater agreement was higher, with a percent agreement of 87.3% ($\kappa = 0.876$). The difference between inter and intra-rater reliability may be explained by difficulties in synchronisation of the coding windows due to different software systems used.

3.4 The use of appropriate electromyographic methodology

The relationship between visually observable changes to movement and their neuromuscular origins is quite complex. Multiple factors determine the eventual outcome of a muscle strategy; a movement is affected by interplay of length of the muscle; the location of attachment to the bone; its inherent strength attributes; the direction of its radiation and even its physiological excitation properties and the combination of muscles involved in the movement. Nikolai Bernstein (1967) was one of the first to describe this phenomenon. Motor tasks, even if automated and rhythmical, are often so complex and changeable that the calculation of the enormous number of possible options to achieve the outcome is too complex to express in a simple mathematical solution. This high number of options originates from the multiple redundant pathways that are available to a given motor system. The central nervous system initially cannot deal with this abundance in the degrees of freedom – but it must be noted that without the multitude of options, it would not be possible to adapt. Bernstein describes a gap between the demands
of the changes in external forces throughout the movement and the movement outcome. The nervous system must be highly flexible to be able to fill this gap: it is required to adjust muscular activation to achieve just the correct amount of force, velocity and direction instantly, particularly as most changes cannot be foreseen in advance. Coordination is therefore “the process of mastering redundant degrees of freedom of the moving organ, in other words, its conversion to a controllable system” (Bernstein, 1967, p. 127), which is done through the creation of functional synergies as a result of practice. In skilled movement, this may be expressed in a more stable EMG signal.

However, newborn babies have limited practice at stepping in an upright position in a gravity environment. Chang et al. (2006) noted that toddlers’ EMG in walking was still highly variable. Even in adults, neuromuscular synergies are flexible (Teulier et al., 2012). The development of walking is characterised by a is a delayed stabilisation of muscle strategies (Teulier et al., 2012, Chang et al., 2006) and it may not be appropriate to expect any structure in the EMG of stepping newborns. However, if the muscle output was determined by a CPG in infants, it would be reflected in a much more predictable pattern, and optimally observable directly after birth. Because of the complexity of the factors determining electromyographic signal, it should be studied with appropriate methodology that can describe predictability and variability. Ideally, it should be compared to behavioural output. The section below will give a detailed account of current problems in infant stepping EMG research and highlight points of best practice for this project.

3.4.1 Optimising data recording

Surface electromyography captures voltage changes caused by muscle contraction via electrodes attached to the skin and is used to study function and coordination (Clarys et al., 2010). Preparation for testing can be time-consuming, as the placement of these electrodes needs to be precise. Infant muscles are small, and their locations are close together. Poor electrode placement may cause meaningful voltage changes to be small, especially compared to the generally present noise. Optimising signal-to-noise ratio can be difficult; noise might be confused for tonic background activity. The scale of infant muscles also makes it more likely that voltage changes from contractions in other muscles are recorded (Lam et al., 2003). This cross-talk is often discussed as a limitation in infant stepping research, but unfortunately there are few robust methods to control for this phenomenon. Some researchers look for occurrences of reciprocal activity as a sign that
cross-talk between these muscles is absent. If one muscle can contract without detection of activity in the other muscle, cross-talk is less likely (Yang et al., 1998a). This is particularly important when coactivity is measured. Interpreting cross-talk for coactivity could lead to finding more coactivity in smaller, weaker muscles.

To further optimise signal transfer, low impedance of the skin is required. Impedance, 'the total electrical opposition of a circuit to the passage of an alternating current through it' (Lawler et al., 1960), can be lowered by cleaning or abrasion of the skin. Baby skin is very sensitive, and sometimes, gentle cleaning methods may not sufficiently lower the skin impedance, leading to poor data (Okamoto et al., 2003). Assaiante et al., (2000) noted that skin impedance may be different for different age groups. It is possible that newborn babies have lower skin impedance because creams and dead skin cells are mostly absent and the subcutaneous fat layer is smaller than for older children and adults, however, there is no quantitative study supporting this idea.

Once data collection commences, further problems may arise at the skin-electrode interface. In dynamic trials, pulling or rubbing of the electrode along the skin might cause discharges that are quite similar to voltage changes due to muscle contraction (Pang and Yang, 2001, Hedberg et al., 2007). In infants, the voltage changes may be so small that motion artefact dominates. Motion artefact can be detected and filtered out, as the frequency components are much lower than those in contraction output. However, in extreme cases it may lead to gaps in the data.

3.4.2 Filtering

Before EMG can be analysed, the raw data requires considerable processing. Signal is generally rectified and filtered. The choice of cut-off frequencies – outside of which the frequencies are attenuated – is often a compromise between noise and artefact reduction and ‘preserving the desired information from the sEMG signal’ (De Luca et al., 2010, p. 1573). De Luca and colleagues advise that the cut-off frequency is set where noise amplitude is likely to surpass the amplitude of the signal (around 400-450 Hz). At the low end of the spectrum, cut-off frequencies need to consider the source of the noise, which include cable sway noise, noise from external devices and movement artefact noise originating from movement between electrode and skin, or skin and muscle (De Luca et al., 2010). These authors recommend a lower cut-off frequency of 20 Hz. However, there are no guidelines on the filtering frequencies of infant EMG. Frequencies ranged from 75-300 Hz (Chang et al., 2006, Teulier et al., 2012, Spencer and Thelen, 2000), although
combinations of high and low pass filters with cut-off frequencies between 10 and 30 Hz are also used (Lam et al., 2003, Pang and Yang, 2002, Ivanenko et al., 2005), sometimes with rectification in between filtering (Dominici et al., 2011). It appears that a wide range of cut-off frequencies is used, and that the level of filtering often depends on the methodology to further process the data: is a high level of detail required (high upper and lower cut-off frequencies), or is the investigator looking for a much more global signal profile (low upper and lower cut-off frequencies)?

3.4.3 Analysis of the EMG

Once EMG is prepared, the analysis can take many forms. Analysis methods often reflect the theoretical background, with extensive pooling - or averaging - of step cycle EMG for those who are looking for common patterns (Yang et al., 1998a, Lam et al., 2003, Yang et al., 2005). Only Dominici et al., (2011) defined variability of EMG traces: ‘the EMG profiles were similar relative to a cycle regardless of its period’ (p. 997). However, measures were only compared between groups of averaged cycles of similar length, and did not reflect true inter-cycle variability. Others found considerable variability for many muscles during the step cycle (Thelen and Cooke, 1987, Teulier et al., 2012). Caution is needed when generating group means through the collapsing of individual data, because ‘this obscures relations that reside at the individual level’ (Vereijken and Thelen, 1997, p. 91). To ensure the validity of the interpretations, methods that use data pooling needs to be accompanied by quantitative measures of variability.

Changes in electrode placement and/or fat distribution (Spencer and Thelen, 2000) and changes in skin impedance (Assaiante et al., 2000) can cause differences that do not reflect changes in the motor pattern in longitudinal design, but even between trials, changes can occur to affect EMG amplitude. Normalisation is generally performed to equalise differences and obtain comparable values for amplitude. In adults, EMG can be normalised by method of maximum voluntary contraction: the signal is calculated as a percentage of this maximum. A maximum voluntary contraction cannot be reliably elicited in infants, and normalisation with other methods may not be completely accurate (Yang and Winter, 1984). Therefore, analysing signal amplitude may be less appropriate in infant stepping research.
3.4.4 Studying coordination with EMG
It is possible to avoid the use of amplitude while maintaining the measurement of muscle contraction. Spencer and Thelen (2000) designed a novel method for an infant reaching study, and converted the signal to an 'on-or-off’ sequence by comparing its amplitude to a threshold. This threshold was based on amplitudinal deviation from background noise level (i.e. the modal amplitude). The analysis focused on the combinations of simultaneously active muscles during reaching. Coordinative muscle strategies were interpreted together with kinematic observations. Chang et al. (2006), Pantall et al. (2012) and Teulier et al. (2012) have applied the same strategy to stepping and walking studies in infants and toddlers. These researchers used a probability analysis, which calculated the likelihood that a muscle was active during different time points of the step cycle. Additionally, they calculated the frequencies in which certain combinations of muscles were active, as well as traditional coactivity values between antagonist muscle pairs. They found a high level of variability and coactivity for all infants, with a low probability of activation that did not vary much across the entire cycle. Muscle contraction became less variable and more defined over time, but not as much as the simultaneous kinematic analysis would have predicted.

3.4.5 EMG analysis summary
The difficulties in collecting, analysing and interpreting the data have often pushed EMG into a qualitatively supportive role of kinematic analysis. Yet, when researchers were careful and designs were simple, interesting contributions were made to science. The analyses most suitable to detect changes in coordination and the development of defined muscle action in stepping omit the use of amplitude and compress the data into an interpretable form without obscuring the role of variability. This thesis has adopted the methods described above, which are sensitive to variability but are still able to interpret neuromuscular changes in combination with kinematic and further quantitative analyses.

3.5 Statistics
Statistical analysis was performed using SPSS (IBM SPSS Statistics versions 19 to 22). In the main analysis, the effects of two factors (condition and arousal level) were explored on 12 (Experiment 1) and seven (Experiment 2) dependent variables. The condition factor consisted of four different treadmill speeds (static, slow, medium and fast) in the first experiment and four different optic flow conditions (no OF, random, congruent and...
faster) in the second experiment. Arousal contained two levels: crying and non-crying, in both experiments.

In Experiment 1, the 12 dependent variables consisted of steps and pumps per second, step percentage, typical and atypical steps per second, typical step percentage, single, parallel, alternated and serial step percentage, cycle duration and stance ratio. In the second experiment, steps and pumps per second, step percentage, and single, parallel, alternated and serial step percentage were examined. Chapter 6 and 7 contain secondary analyses with additional dependent variables, and are described in detail in the chapters. Because of consistent violation of assumptions for parametric testing, the majority of the tests were nonparametric (Friedman and Wilcoxon Signed-rank tests). The following section clarify and justify the choice of the statistical tests throughout the chapters.

3.5.1 Testing for normal distribution of the data

Prior to statistical analysis, the distributions of all data sets were tested for normality using Shapiro-Wilk tests. In Experiment 1 (Chapter 4), none of the variables were normally distributed in the by-condition analysis, and only 42% of the data was normally distributed in the by-arousal analysis. Across both factors, the data showed evidence of considerable positive skew - exceeding 1 in 14 cases and 2 in two cases - and with a negative value exceeding -1 in two variables. In Experiment 2, none of the variables in the by-condition analysis were normally distributed, and 43% of the data analysed in the by-arousal analysis was not normally distributed. Across the two factors, the skew value exceeded 1 in four variables and was lower than -1 in two cases. In Chapter 6, the kinematics were normally distributed in some variables, yet, in 50% of cases, distributions were not normal. In the EMG analysis (Chapter 7), distributions of the tested variables were unpredictable across all variables and both experiments. In the probability analysis in Experiment 1, for example, the probability of activation (POA) was normally distributed for all time windows in the Rectus Femoris alone, and only during the stance phase. In Experiment 2, the POA of the Rectus Femoris was normally distributed during the swing in the movement by optic flow condition, and the POA of all muscles were distributed normally during the swing phase in the movement by arousal analysis. Approximately half the variables showed non-normal data distributions.

The lack of normality in the data distributions in this project was most likely caused by skew. Most of the distributions were unimodal, and kurtosis was large on a small number of occasions (exceeding 2 or -2). Skew was most often positive, with longer tails on the
right-hand side of the curve. This may be normal for newborn behaviour. Square root transformations and logarithmic transformations were attempted to normalise the data distributions. However, even after transformation the data did not fit a normal distribution, therefore nonparametric testing was deemed most correct in this project.

3.5.2 Tests used in the main analysis

The nonparametric equivalent to the repeated measures analysis of variance - the Friedman test - was used to investigate statistical differences across four treadmill speed conditions in Chapter 4 and the four optic flow conditions in Chapter 5. Because the Friedman test does not automatically detect between which conditions a difference occurred, planned post hoc tests were used (Wilcoxon Signed-rank tests, a nonparametric equivalent of the paired samples T-test), and Bonferroni-Holm corrections (Holm, 1979) were made to correct for multiple comparisons. Wilcoxon Signed-rank tests were also used to examine the differences between arousal levels in both experiments. The additional analyses in Chapter 6 and 7 were also nonparametric, with the Friedman and Wilcoxon Signed-rank test procedure to detect significant differences between conditions and Wilcoxon Signed-rank tests to detect significant differences between arousal levels. The complete results are displayed in the tables, with significant differences highlighted in the text of the chapters.

3.5.3 Additional tests

In both studies, three more separate tests were performed to examine the influence of condition or trial order on arousal, and to examine whether trial order influenced movement and step rate. Because the arousal variable was normally distributed, it was possible to utilise parametric tests for the first two analyses: repeated measures analyses of variance (RM ANOVAs) were performed. However, movement and step rates were not normally distributed and Friedman tests were performed to assess whether trial sequence (first, second, third or fourth trial) affected overall movement or step rate. Additionally, in Chapter 5, an analysis of ‘looking’ time, or the time in which infants had their eyes open, was conducted. The dependent variable, eye opening time, was normally distributed and an RM ANOVA was used to assess if condition affected eye opening time. Finally, a between-experiment analysis investigated the differences between the two experiments by examining some variables from the comparable conditions (the slow treadmill speed condition from Experiment 1 and the no optic flow condition in
Experiment 2): in Chapter 7, activity ratio was compared and in Chapter 8, average arousal level was compared. For these analyses, the data was normally distributed, and therefore, Student’s t-tests were used.

3.5.4 Interaction
The multifactorial design of the experiments warranted testing for interaction. Even though there was no difference in arousal level across the different conditions, it is possible that arousal level affected movement quantity and quality within individuals. If parametric testing had been possible, a two-way repeated measures analysis of variance may have been used in a 4 (condition) x 2 (arousal) design, however, in nonparametric testing it is not possible to automatically assess interaction effects. Alternatives are available to assess interaction in nonparametric testing, for example, the adjusted rank transform test, which is powerful at small sample sizes (Sawilowski 1990, Leys and Schumann 2010). However, only where data are complete across both factors is it possible to perform tests of interaction. In the current project, not every newborn displayed both crying and non-crying states. For example, in Experiment 1, 21 infants produced measures for steps per second, pumps per second and step percentage, but only three infants had periods of crying and non-crying in each of the conditions. Therefore, the data could not be assessed for interaction. When reading this work, consider that it is possible that arousal level affected the response to condition.

3.5.5 Type I error
When many research questions or variables are examined in one experiment, the chance that one significant difference is found coincidentally increases linearly with the number of tests performed. This multiple testing problem occurs because the alpha level (α) describes the probability that the observed effect was due to chance. With a p-value lower than an α of 0.05, there is less than 5% possibility that the difference was simply due to chance. Theoretically, in twenty observations, one of the null hypotheses will be rejected falsely (type I error), with cumulative effects as the number of tests grows. To maintain α throughout an experiment with multiple tests, several methods of correction are possible (Sinclair et al., 2013, Howell, 2012). In this project, multiple comparisons within an independent test (i.e., post hoc tests), were corrected for with the Bonferroni-Holm correction (Holm 1979). However, these correcting methods also increase the possibility
that type II error occurs - that a significant difference is dismissed where an effect was truly present.

In the first experiment (Chapter 4), correction of the main tests with the Bonferroni-Holm method would have resulted in the exclusion of all six significant differences in the movement by condition analysis, and three out of the eight effects would not be significant in the movement by arousal analysis. In the second experiment, the only significant difference in the movement by condition analysis would remained significant after correction, but only one of three effects in the movement by arousal analysis. No significant differences would have been observable in Chapter 7 (27 separate tests) or Chapter 8 (110 separate tests for two experiments).

Sinclair et al. (2013) make a case for abolishing all multiple comparisons corrections because firstly, there might be alternative solutions to the null-hypothesis, and ‘to pay a penalty for making additional observations should be considered unacceptable to any scientist’ (Sinclair et al., 2013, p. 19). Secondly, correction may over-inflate the risk of type II errors in explorative studies: their purpose is to explore, not to test one distinct null-hypothesis. As the current project is of an explorative nature, correction of the multiplicity problem was not deemed justified.

3.5.6 Power

Power is defined as “the probability of correctly rejecting a false null-hypothesis when a particular alternative hypothesis is true” (Howell, 2012, p. 203), or in other words, the capability to find an effect when it is truly there. Power depends on alpha level, effect size and sample size (Button et al., 2013). In the current experimental design, minimal sample size was calculated based on with G*Power, a freeware ‘general stand-alone power analysis program for statistical tests commonly used in social and behavioral research’ (Faul et al., 2007, p. 175). Entry values were based on a previous study examining newborn air stepping (Barbu-Roth et al. 2009). Alpha level was set at 0.05, effect size at 0.412 (as calculated from the $\eta^2$ value given in Barbu-Roth et al., 2009), and power at 0.8. Minimal required sample size was computed as 24 for either study.
Chapter 4:  
TREADMILL STIMULATION IMPROVES NEWBORN STEPPING
4.1 Abstract

To shed further light on infant stepping, we investigated whether newborns could step on a treadmill and adapt their steps to graded velocities. Twenty-one newborns (mean age three (±1.8) days) were supported for 60 second trials on a treadmill that was static or moved at 0.134, 0.172, or 0.234 m • sec⁻¹. Video analysis revealed that newborns made more real steps than in-place ‘pumps’ on the moving treadmill than on the static treadmill and made more real steps at 0.172 than 0.234 m • sec⁻¹. While the treadmill had no effect on arousal, stepping increased and showed higher quality and coordination across conditions when infants were crying. These findings suggest that treadmill interventions currently used to promote the development of independent locomotion in infants at risk of delay could begin at birth. Further investigation is needed to establish the optimal conditions for newborn treadmill stepping and to specify how arousal affects step rate, quality, and coordination.

Keywords: locomotion; neonate; perceptual-motor development; walking

4.2 Introduction

4.2.1 Stepping in human newborns

Directly after birth, human infants are capable of producing stepping movements on a solid surface when an adult holds them under the armpits in an upright position, tilts them forward, and provides continuous support as they move forward (McGraw, 1940). Although functional features of stepping at birth such as propulsion and forward translation have not been quantified in detail, infant steps often show “march-like” characteristics (Thelen, 1986b) with simultaneous and extensive hip and knee flexion and minimal step length (Forssberg, 1985). A link between newborn stepping and later functional walking was proposed by André-Thomas and Autgaerden (1966) and Zelazo, Zelazo, and Kolb (1972), who found that newborn infants who trained daily increased stepping frequency and attained independent walking earlier. When not trained, the behaviour disappears at approximately two months and returns towards the end of the first year, before an infant starts to walk independently (McGraw, 1932). Thelen and Fisher (1982) and Thelen, Fisher and Ridley-Johnson (1984) suggested that stepping disappears because infants gain weight rapidly in their first two months of life and become less able to lift their legs against gravity. Thelen also found that the behaviour could be
re-elicited after the age of two months by supporting infants over a motorized treadmill (1986b). The treadmill appeared to decrease variability and elicit more complex patterns of intra and interlimb coordination, suggesting that treadmill use shifted the infants into a more advanced, stable movement pattern (Thelen and Ulrich, 1991).

4.2.2 Treadmill training
The view that specific motor experience will strengthen existing motor patterns (Vereijken and Thelen, 1997) led to the successful application of treadmills in training paradigms for infants at risk of developing locomotor delays. Regular daily treadmill training, started at a few months of age, increased stepping ability in typically developing infants (Vereijken and Thelen, 1997) and brought forward the onset of independent walking in infants with motor deficiencies (Bodkin et al., 2003, Ulrich et al., 2001). In particular, treadmill-trained infants with Down syndrome retained qualitative gait improvements after the onset of walking, especially in terms of step length (Wu et al., 2007). However, these training studies included only older infants between five and 12 months of age who no longer exhibited the newborn stepping response (Bodkin et al., 2003, Ulrich et al., 2008, Ulrich et al., 2001). Would training have been more effective if started earlier, when stepping could still be easily evoked?

As interventions are more beneficial when the neuromuscular system is at its most plastic stage (Ulrich, 2010), an important training opportunity may exist immediately after birth (Barbu-Roth et al., 2013, Teulier et al., 2013). Starting treadmill training earlier may 1) offer extra time during this critical window of enhanced neural plasticity; and 2) utilize newborns’ ability to step before it attenuates, which may be essential for at-risk infants who naturally step less. Newborns’ high level of adaptability to their environment and ability to modify step frequency to visual stimuli simulating their forward displacement (Barbu-Roth et al., 2009, Barbu-Roth et al., 2013) suggests that newborns may also respond to a moving treadmill surface by taking more steps and producing higher quality steps. To date, no study on treadmill stepping has quantified newborn responses. The main goal of this study was to investigate whether newborns would change their stepping behaviour if their legs were treadmill driven. We examined whether a moving treadmill would increase step frequency and step quality and whether newborns could adapt their step characteristics to different treadmill velocities. A positive result to any of these questions would suggest that treadmill interventions for infants at risk for developmental delay could be initiated at birth.
4.2.3 The role of arousal

Arousal, an often-overlooked confounding factor in the infant stepping literature, was also investigated. It is well known that newborn infants are easily aroused, and a positive relationship between arousal level and stimulus response (Prechtl, 1974) and movement frequency and vigour has been shown (Hadders-Algra et al., 1993). Although a positive relationship between arousal and stepping has been reported (e.g., Thelen et al., 1982, Thelen et al., 1984), the strength of the relationship is unclear, particularly in newborns, and the arousal-stepping relation has rarely been examined on the treadmill (cf., Thelen and Ulrich, 1991). It was hypothesised that the newborns would exhibit more stepping movements, higher quality steps, and more alternating steps in the moving treadmill conditions. However, it was unclear whether newborns would adjust their step rates or step characteristics to the different treadmill velocities. Additionally, arousal should increase stepping rate but it was uncertain if arousal would affect step quality and coordination.

4.3 Methods

4.3.1 Participants

Twenty-eight newborns were recruited from the Port Royal maternity hospital in Paris. Only healthy infants with an Apgar score of 10 at the fifth minute, a minimum birth weight of 2500 grams, a minimum term of 38 weeks, and uncomplicated delivery were selected. Seven infants were excluded from the final sample (see methods Chapter 3.1). Twenty-one infants were included in the analysis (11 male, 10 female; weight 3254.9 (±402.5) grams; age three (±1.8) days; mean term 39.5 (±1.1) weeks). Ethical approval was obtained from the ethics medical board of Paris-Ile de France and parents and caregivers gave informed consent for infant participation.

4.3.2 Experimental set-up and materials

An infant-sized treadmill (belt surface 0.31m x 0.59 m) was placed on a 1m x 1m x 0.46m (l x w x h) table. Two digital mini DV cameras (Sony DCR-HC26) captured infant movements at 30 Hz; one was placed perpendicular to the center of the treadmill at 1.275m height, the second camera was placed in front of the treadmill at 0.6m distance from the center of the treadmill (Figure 4).
4.3.3 Procedure

Infants were tested after feeding and when they were awake and not crying. They were undressed to their undergarment or diaper and supported by the experimenter under the armpits above the treadmill surface (in an upright position, angled slightly forwards with the head and neck supported by the thumbs and the index finger). Infants were tested in four randomly ordered 60 second trials with different treadmill speed conditions. For condition 1 (static), the treadmill belt was static. For condition 2–4, the treadmill speed was raised from 0.134 m • sec⁻¹ (slow) to 0.172 m • sec⁻¹ (medium) and 0.234 m • sec⁻¹ (fast). These speeds were selected based on other treadmill experiments testing one to two-month-old infants (Moerchen and Saeed, 2012, Thelen and Ulrich, 1991, Teulier et al., 2012) and air stepping experiments testing three-day-old infants (Barbu-Roth et al., 2009, Barbu-Roth et al., 2013). In the static condition, the newborn was supported in place at the base of the treadmill and translated forward when a step was taken to ensure the trunk remained tilted slightly forward. If the newborn reached the front end of the treadmill, he or she was repositioned at the base. In the moving conditions, the infant was initially positioned at the center of the treadmill and then repositioned at the front end of the treadmill if he or she had not responded to the treadmill for more than 10 seconds, so
that the hip was more likely to move through flexion and extension. Due to the amount of care needed when testing newborns, complete blinding of the experimenter was impossible; however, the experimenter was unable to distinguish between different treadmill speeds except the static condition. If infants showed signs of high distress, e.g., desperate crying without pauses, or if the parents indicated their infant was too stressed to continue, they were removed from the treadmill before the end of the trial. As a result, some trials were shorter. Trials shorter than 30 seconds in total were discarded from the analysis because they were considered too short to represent the newborn’s potential to exhibit stepping (see Appendix B for a complete overview of testing times). Missing trials were not repeated because the neonates were only in an optimal condition for testing for a brief period of time.

4.3.4 Data reduction

4.3.4.1 Movement coding
Videos captured with the sagittal view camera were imported into Siliconcoach software (Siliconcoach Ltd, Dunedin, New Zealand version 7-0-2-2) to code infant movement. All data were initially coded by one experimenter and 11% of the trials were re-coded by a second experimenter. The average inter-rater agreement across all movement parameters was 86.1% (see Chapter 3.3.4 for the reliability analysis details). Periods in which excessive tester movement (e.g., to comfort the infant) were observed were excluded from the analysis because they led to movements that were not generated by the infant (see Appendix B for final trial lengths).

Two categories of movements were identified from the variety of newborn leg motions: pumps and steps (for a more detailed description, see Chapter 3.3.2). A pump was defined as a vertical flexion–extension cycle that displayed no locomotive potential in the upright position. The foot travelled forward less than half a foot-length during the swing phase. A step was defined as a flexion–extension cycle in which the foot travelled forward more than half a foot-length during the swing phase. Steps were further classified based on their quality. Typical steps were those in which there were clearly observable stance and swing phases, the foot travelled forward more than a foot length during the swing phase, and the foot touched down in front of the hip at the end of the swing phase. Atypical steps were defined as steps that had an interrupted or shuffling stance phase, or the foot travelled
backwards less than a foot-length - but more than half a foot-length - during the stance phase, or the foot touched down behind the hip at the end of the swing phase.

Next, inter-limb coordination was determined for typical and atypical steps using criteria established by Thelen and Ulrich (1991). Unilateral steps on one leg were labelled as single steps. Parallel steps were initiated between the beginning and 20% of a step cycle on the opposite leg. Alternating steps were initiated within 20–80% of a step cycle on the opposite leg. Serial steps - a new category by Barbu-Roth and colleagues (2013) - were initiated after 80% of a step cycle on the opposite leg and up to 1 second after the end of the step cycle on the opposite leg. Finally, temporal characteristics of the step cycle were defined under the cycle analysis. Step cycle time pertained to the time between consecutive toe-offs. Stance ratio was the time the foot was on the surface divided by the step cycle time (e.g., a stance of 0.5 second during a cycle of 1 second gives a stance ratio of 50%).

4.3.4.2 Arousal coding.
Frontal videos were used to code level of arousal. Thelen’s six-point scale adaptation (Thelen et al., 1984) of Prechtl’s behavioural states (Prechtl, 1974) was modified to suit the current project. Arousal was sampled at five second consecutive intervals to exclude infants who were under-aroused (state 1 and 2), to allow precise mapping between stepping and arousal during active arousal states (4, 5 and 6), and to track changes in behavioural state throughout the experiment. We examined whether treadmill condition and testing time influenced arousal and we examined stepping characteristics when infants were in the crying states (state 5 and 6) versus the non-crying alert state (state 4). Arousal state was identified for each step and pump, based on the code assigned to the five second interval in which the majority of the step/pump occurred. The first three states were excluded: during state 1, newborns were asleep and made no movements. This state was not observed in the final sample. No steps or pumps were made during state 2: movement was severely reduced and consisted of startles and some slow movement in the extremities. During state 3, infants typically are quietly alert with their eyes open, but do not move. However, there was a minimal number of steps that fell during state 3 windows, but only in transition periods to higher arousal states. These steps were not considered truly reflective of the characteristics of state 3 and were also excluded. Two experimenters individually coded all trials with an intra-rater percentage agreement value of 87.3% (see Chapter 3.3.4 for the reliability analysis details).
4.3.5 Statistical analysis
For the main analysis, Shapiro-Wilk tests showed that the data were not normally distributed. Therefore, nonparametric tests were used to examine the effect of treadmill speed and arousal. For the repeated measure of treadmill speed, a Friedman test was used on step and pump rate, step percentage, typical and atypical step rate, typical step percentage, single, parallel, alternating and serial step percentage, cycle duration and stance ratio. Significance level was set at 0.05. Because Friedman tests cannot determine between which condition the differences occurred, post hoc effects were examined by testing between every possible combination of treadmill speed pairs, using Wilcoxon Signed-rank tests. Alpha levels of these analyses were adjusted to correct for multiple comparisons (Holm 1979).
To investigate the effect of different arousal states, Wilcoxon Signed-rank tests were used on the same variables (step and pump rate, etc.). If infants did not step in one of the conditions, they were excluded from the step quality and coordination analyses. Consequently, the sample sizes for these analyses were smaller than those for the movement type analyses. The sample sizes for the cycle analyses were smallest because the stance phase could not be determined for both legs during parallel steps. The interaction between the two factors could not be looked at directly, therefore repeated measures analyses of variance (RM ANOVAs, as the data were normally distributed in this analysis) were run to test if arousal was equally distributed across all conditions and across all trials. For more details on the statistics and its reporting, see Chapter 3.3.

4.4. Results

4.4.1 The effects of treadmill speed on stepping
The results for the effects of treadmill speed are reflected in Table 2.
Table 2: Step parameters by treadmill speed, with descriptive and inferential statistics for the parameters measured in the static (0 m • sec⁻¹), slow (0.134 m • sec⁻¹), medium (0.172 m • sec⁻¹), and fast (0.234 m • sec⁻¹) treadmill conditions. Note that the percentages do not sum to 100%, since medians and not means are reported. The analyses in some categories contain smaller sample sizes due to incomplete data sets that were excluded in the nonparametric statistical tests; some infants did not move or step in each category (see Appendix B for a complete overview of excluded data).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>χ²</th>
<th>N</th>
<th>P-value</th>
<th>Medians</th>
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<td></td>
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<td></td>
<td>Static</td>
</tr>
<tr>
<td>Leg movement category</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steps (sec⁻¹)</td>
<td>34.435</td>
<td>21</td>
<td>0.001</td>
<td>0.018bcd</td>
</tr>
<tr>
<td>Pumps (sec⁻¹)</td>
<td>1.019</td>
<td>21</td>
<td>0.797</td>
<td>0.072</td>
</tr>
<tr>
<td>Steps (%)</td>
<td>27.000</td>
<td>21</td>
<td></td>
<td>0.000bcd</td>
</tr>
<tr>
<td>Typical step (sec⁻¹)</td>
<td>29.807</td>
<td>21</td>
<td>0.001</td>
<td>0.000bcd</td>
</tr>
<tr>
<td>Atypical step (sec⁻¹)</td>
<td>27.849</td>
<td>21</td>
<td></td>
<td>0.001bcd</td>
</tr>
<tr>
<td>Typical step (%)</td>
<td>4.570</td>
<td>12</td>
<td>0.206</td>
<td>27.92</td>
</tr>
<tr>
<td>Step quality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single step (%)</td>
<td>11.067</td>
<td>12</td>
<td>0.011</td>
<td>45.00</td>
</tr>
<tr>
<td>Parallel step (%)</td>
<td>15.991</td>
<td>12</td>
<td>0.001</td>
<td>0.000bcd</td>
</tr>
<tr>
<td>Alternated step (%)</td>
<td>7.769</td>
<td>12</td>
<td>0.051</td>
<td>33.33</td>
</tr>
<tr>
<td>Serial step (%)</td>
<td>1.946</td>
<td>12</td>
<td>0.584</td>
<td>0.00</td>
</tr>
<tr>
<td>Step cycle analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle duration (s)</td>
<td>11.000</td>
<td>9</td>
<td>0.012</td>
<td>2.950bcd</td>
</tr>
<tr>
<td>Stance ratio (%)</td>
<td>11.933</td>
<td>9</td>
<td>0.008</td>
<td>67.08d</td>
</tr>
</tbody>
</table>

Note: Superscript letters indicate significance within categories: a, significantly different from static; b, significantly different from slow; c, significantly different from medium; d, significantly different from fast.

Leg movement category

The number of steps per second ranged from 0.018 in the static condition to 0.259 in the medium condition (Figure 5). The Friedman test was significant ($\chi^2 = 34.435, p = 0.001$). The post hoc test revealed that more steps per second were taken in the moving conditions than in the static condition and less were taken in the fast (0.170) condition than in the medium condition. The number of pumps per second did not differ across conditions, however, step percentage (calculated as a percentage of total movements, i.e., steps and pumps) did differ ($\chi^2 = 7.000, p = 0.001$), with significantly higher step percentages in the slow (79.29%), medium (83.33%), and fast (78.17%) conditions than in the static (19.62%) condition (Figure 6).
The number of typical steps per second was significantly different across conditions ($\chi^2 = 29.807$, $p = 0.001$). Fewer typical steps per second were found in the static condition (0) compared to the slow (0.065), medium (0.102), and fast (0.043) conditions and infants produced significantly more typical steps in the medium than in the fast condition (Figure 7). The number of atypical steps per second was also significantly different across conditions ($\chi^2 = 27.849$, $p = 0.001$), with significantly more in the fast (0.123) than in the static (0.036), slow (0.018) and medium (0.117) conditions. The medium and static
conditions were also significantly different. The percentage of typical steps (calculated as a percentage of total steps) did not differ across conditions (Figure 8).

Figure 7: The effects of treadmill speed on step quality

![Box plot showing the effects of treadmill speed on step quality.](image)

Figure 8: The effects of treadmill speed on typical step percentage

![Box plot showing the effects of treadmill speed on typical step percentage.](image)

**Step coordination**

The only significant difference was found in the parallel stepping category ($\chi^2 = 15.991$, $p = 0.001$), with a significantly lower percentage of parallel steps in the static condition...
(0%) than in the slow (32.18%), medium (43.65%), and fast (26.62%) conditions (Figure 9a). The Friedman test found a difference in the single step category, but this was not significant after correction for multiple comparisons.

![Figure 9: The effects of treadmill speed on step coordination (a: single and parallel steps; b: alternated and serial steps)](image)

**Figure 9:** The effects of treadmill speed on step coordination (a: single and parallel steps; b: alternated and serial steps)

**Cycle analysis**

Step cycle duration was significantly different between conditions ($\chi^2 = 11.000$, $p = 0.012$), with longer duration in the static condition (2.95 seconds) compared to the medium (1.733 seconds), and fast (1.685 seconds) conditions (Figure 10a). Stance ratio was also significantly different across conditions ($\chi^2 = 11.933$, $p = 0.008$). It was
significantly greater in the static (67.08%) than in the fast (48.44%) condition (Figure 10b).

![Box plots showing cycle duration and stance ratio across different treadmill speeds.](image)

**Figure 10:** The effects of treadmill speed on the step cycle (a: cycle duration and b: stance ratio)

### 4.4.2 The effects of arousal level on stepping

A repeated measures ANOVA showed that mean arousal level did not change between each consecutive trial. Means ranged from 3.81 in trial 3 to 4.20 in trial 1, $F_{3,60}=1.215$, $p = 0.312$. Arousal did not change as a function of condition, with means of 3.81 when the treadmill was fast compared to 4.30 when the treadmill was at medium speed, $F_{3,20} =$
1.362, p = 0.263. Additionally, Friedman tests showed that the number of movements (χ² = 0.449, p = 0.930) or steps per second (χ² = 1.096, p = 0.778) did not increase across testing time, therefore, there was no evidence for learning or fatigue effects in this study.

The main results are described in Table 3.

Table 3: Step parameters by arousal state, with descriptive and inferential statistics for the parameters measured when infants were crying or not crying. Note that the percentages do not sum to 100%, since medians and not means are reported. The analyses in some categories contain smaller sample sizes due to incomplete data sets that were excluded in the nonparametric statistical tests; some infants did not move or step in each category (see Appendix B for a complete overview of excluded data).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Z-value</th>
<th>N</th>
<th>P-value</th>
<th>Medians</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Crying</td>
<td>Non-crying</td>
</tr>
<tr>
<td><strong>Leg movement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steps (sec⁻¹)</td>
<td>-2.296</td>
<td>17</td>
<td><strong>0.022</strong></td>
<td>0.269</td>
<td>0.151</td>
</tr>
<tr>
<td>Pumps (sec⁻¹)</td>
<td>-2.533</td>
<td>17</td>
<td><strong>0.011</strong></td>
<td>0.137</td>
<td>0.057</td>
</tr>
<tr>
<td>Steps (%)</td>
<td>-0.795</td>
<td>17</td>
<td>0.427</td>
<td>65.48</td>
<td>66.67</td>
</tr>
<tr>
<td><strong>Step quality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typical step (sec⁻¹)</td>
<td>-2.580</td>
<td>17</td>
<td><strong>0.010</strong></td>
<td>0.117</td>
<td>0.045</td>
</tr>
<tr>
<td>Atypical step (sec⁻¹)</td>
<td>-0.828</td>
<td>17</td>
<td>0.407</td>
<td>0.115</td>
<td>0.101</td>
</tr>
<tr>
<td>Typical step (%)</td>
<td>-2.272</td>
<td>16</td>
<td><strong>0.023</strong></td>
<td>52.27</td>
<td>33.33</td>
</tr>
<tr>
<td><strong>Step coordination</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single step (%)</td>
<td>-1.603</td>
<td>16</td>
<td>0.109</td>
<td>31.48</td>
<td>50.00</td>
</tr>
<tr>
<td>Parallel step (%)</td>
<td>-1.363</td>
<td>16</td>
<td>0.173</td>
<td>35.42</td>
<td>25.00</td>
</tr>
<tr>
<td>Alternated step (%)</td>
<td>-1.961</td>
<td>16</td>
<td><strong>0.050</strong></td>
<td>16.08</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Step cycle analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle duration (s)</td>
<td>-1.293</td>
<td>15</td>
<td>0.196</td>
<td>2.027</td>
<td>1.652</td>
</tr>
<tr>
<td>Stance ratio (%)</td>
<td>-0.804</td>
<td>15</td>
<td>0.422</td>
<td>44.39</td>
<td>51.82</td>
</tr>
</tbody>
</table>

Note that small sample sizes were common because infants did not always make steps in each arousal state. Additionally, the sample sizes for the cycle analyses were smallest because the stance phase could not be determined for both legs during parallel steps.

**Leg movement category**

The Wilcoxon test showed a significant difference in the number of steps per second when newborns were crying (0.269) compared to when they were not crying (0.151; Z = -2.296, p = 0.022) and a significant difference in pumps per second during crying (0.137) compared to non-crying (0.057; Z = -2.533, p = 0.011, see Figure 11). Figure 12 shows that no significant differences were found in the percentage of steps (calculated as a percentage of total movements, i.e., steps and pumps).
The Wilcoxon test showed a significant difference in the number of typical steps per second during crying (0.117) compared to non-crying (0.045; \(Z = -2.580, p = 0.010\), see Figure 13). No significant difference was found for the number of atypical steps per second, but the percentage of typical steps (calculated as a percentage of total steps) was
higher during crying (52.27%) than non-crying (33.33%; Z = -2.272, p = 0.023, see Figure 14).

Figure 13: The effect of arousal on step quality

![Figure 13: The effect of arousal on step quality](image)

Figure 14: The effect of arousal on typical step percentage

![Figure 14: The effect of arousal on typical step percentage](image)

**Step coordination**

The Wilcoxon test showed a significant difference in the percentage of alternated steps during crying (16.08%) compared to non-crying (0%) (Z = -1.961, p = 0.05, see Figure 15b). Additionally, a higher percentage of serial steps was found when infants were not
crying (10.35%) compared to when they were crying (0%) ($Z = -2.134$, $p = 0.033$, see Figure 15b).

![Figure 15: The effect of arousal on step coordination](image)

**Figure 15: The effect of arousal on step coordination**

**Cycle analysis**

Figure 16a shows that cycle durations ranged from median 1.652 seconds when infants were crying, to median 2.027 seconds when infants were not crying, however, the
difference was not significant \( Z = -1.293, p = 0.196 \). Stance ratios ranged from median 44.39% for steps made when not crying to 51.82% when steps were made when crying (Figure 16b). This result was also not significant \( (Z = -0.804, p = 0.422) \).

Figure 16: The effect of arousal on cycle duration and stance ratio
4.5 Discussion

4.5.1 Treadmill stimulation improves stepping
This study shows for the first time that a moving treadmill can facilitate stepping in newborns. Consistent with expectations, significantly more steps, but not pumps, and a higher percentage of steps (relative to pumps) were taken in the moving treadmill conditions than in the static condition. In addition, the differences between the static and moving conditions were more pronounced for typical steps than atypical steps. The tendency to pump in the static condition and step in the moving conditions may be explained by placing the behaviour into a newborn context. Flexion-extension cycles of the upper and lower extremities can be observed in utero (Birnholz et al., 1978) and consequently, newborns have considerable experience with in-place flexion-extension cycles (pumps). When the dynamic treadmill surface is introduced, infants are exposed to a novel environment. Passive movement of the legs may stimulate the forward swing via a relationship between limb load and extensor burst duration (Yang et al., 1998a) or a flexion response to hip flexor tension or hip position (Thelen and Ulrich, 1991), changing the characteristics of the newborn’s natural pumping behaviour to forward stepping. Therefore, the treadmill offers a change of task constraints that allows more frequent and advanced behaviour to emerge. The absence of parallel steps in the static condition may be linked to intra-uterine habituation, as Robinson et al. (2006) have observed that simultaneous limb extension in the womb takes place at a higher energetic cost, reinforcing alternation intrinsically. A newborn’s natural state may favour alternation; however, when a very strong stimulus such as that provided by a treadmill is introduced, very young infants tend to respond with an inability to dissociate joints and limbs (Thelen and Cooke, 1987, Teulier et al., 2009). Indeed, Thelen and Ulrich (1991) found a reduction in parallel stepping on the treadmill with increasing age.

4.5.2 Step cycle duration is passively adaptable to treadmill speed
Newborns stepped faster on the moving treadmill than on the static treadmill. This result was congruent with reports in older infants. For example, Yang et al. (1998a) showed shorter step cycle durations for faster speeds in 10 days to 10-month-old infants and Thelen and Ulrich (1991) found similar effects in one to seven-month-old babies. In mature walking, faster speeds are achieved by shortening the cycle duration through a decrease in stance time while swing time remains constant (Pearson, 1976). On the
treadmill, shorter stance phases may be caused by earlier triggering of the swing phase based on the location of the lower leg, suggesting proprioceptive sensitivity in the stepping response directly after birth.

However, Yang et al. (1998a) reported that the cycle duration-treadmill speed relationship fitted a power function and proposed that ‘circuitry generating the alternate stepping movements is well developed at birth’ (p. 935). Conversely, the current study observed no strong linear relation between speed and cycle duration ($r^2 = 0.3$) and found considerable response variability within and between infants, suggesting that stepping emerges from multiple organismic and environmental factors in addition to innate neural circuitry. The difference may result from Yang’s collapsing of results across a considerable range of ages (10 days-10 months) or because the study did not focus on infants’ adaptability directly after birth (e.g., one to three days). Our findings are more consistent with Thelen and Ulrich’s (1991) suggestion that a linear relationship between speed and cycle duration ‘emerges with increasing age’ (p. 64) and that movement experience improves infants’ sensitivity and responsiveness to treadmill speed. Correspondingly, we did not observe a linear increase in step rate in response to faster treadmill speeds, as more steps were produced in the medium condition than in the fast condition. A curve-fitting analysis showed that the relationship between treadmill speed and step rate fit a quadratic, inverted-U relationship better than a linear trend ($F = 18.872$ compared to $F = 9.913$), suggesting that newborn stepping is not simply a function of tactile stimulation. In this relationship, performance increases with increased stimulus, but after reaching the optimum, diminishing returns are observed. In this study, it might illustrate that high treadmill speeds become too fast to reliably elicit a response in newborns.

4.5.3 Arousal affects step and pump frequency, step quality and coordination

Tactile stimulation is not the only factor that influences infant stepping. Anthropometric characteristics (Thelen et al., 1984), vision (Barbu-Roth et al., 2009, Barbu-Roth et al., 2013) and odour (Varendi and Porter, 2001) can affect newborn stepping. A relationship between arousal and infant movement also exists (Prechtl, 1974, Thelen et al., 1982, Thelen and Ulrich, 1991). Infants tend to move more vigorously (Hadders-Algra et al., 1993) and respond more strongly to reflex stimuli when aroused (Vecchierini-Blineau and Guihneuc, 1982). In this study, step and pump frequency and step quality increased when infants were crying, regardless of treadmill speed. When more aroused, newborns
showed a smooth and defined stance-phase and placed their stance foot further forwards. This suggests an ability to move the leg against gravity and commit more weight to the stance leg. It is possible that muscle force increased through improved membrane excitability in aroused states (Thelen et al., 1984, Vecchierini-Blineau and Guiheuneuc, 1982). Significant changes in coordination also occurred with increased arousal as evidenced by an increase in alternating steps. Similar results were found in spinal cats in which the arousal system had been pharmaceutically influenced (Rossignol, 1996b). After stimulation of the noradrenergic system, cats showed stereotypical, automated alternated hindlimb stepping in the absence of cerebral control. Tighter inter-limb coupling may indicate increased response automaticity.

Does arousal improve muscle strength and/or generate automated stepping? Deeper investigation of muscle variability is required to answer this question. Dominici et al. (2011) reported a high correlation coefficient for EMG patterns in newborns between cycles of different length, but Teulier et al. (2012) observed considerable variability in one-month old infants. Neither study recorded arousal. Therefore, detailed examination of how arousal affects activation pattern variability in newborns may provide new information.

One last comment should be made on the influence of arousal. Approximately 25% (seven newborns) of the sample was excluded from the analysis due to over and under-arousal and failure to step, raising questions on the feasibility of treadmill training at birth. However, note that these criteria did not purely reflect newborns’ ability to respond to the treadmill: it mainly showed which infants were temporarily in an inappropriate behavioural state for testing. Only one newborn was excluded due to over-arousal, suggesting that most newborns will tolerate stepping on the treadmill directly after birth. Furthermore, four excluded newborns showed consistently low arousal levels, suggesting that the treadmill did not generically cause distress. Only two excluded infants showed very low response rates without being drowsy or asleep. While it is possible that some infants do not respond strongly to the testing, or dislike the experience, it is not possible to deduct the feasibility of treadmill training at birth from this study. It is entirely possible that factors outside the treadmill caused the high arousal levels in the one over-aroused infant, or indeed the lack of stepping in the two low responders. Testing at a different time may have given completely different results. Nonetheless, it does highlight the importance of judicious session timing if the treadmill was used in daily training.
4.6 Conclusion

In conclusion, a moving treadmill can facilitate newborn stepping, suggesting that the treadmill might be used at birth to enhance locomotor experience in infants at risk for developmental delay. We acknowledge that response rates can be lower in at-risk infants than in our typically-developing cohort and so more research is clearly needed to establish the clinical value of the treadmill paradigm for at-risk newborns. More research is also needed to clarify the role of arousal on step rate and quality in typically-developing and at-risk infants.

4.7 Note at reference ¹

The arousal coding method used in this study was compared to the crying time coding method in Barbu-Roth et al. (2009, 2013); all trials were coded using both systems and a Pearson product–moment correlation showed a strong positive relationship ($r^2 = 0.93$, $p = 0.001$) between methodologies.
Chapter 5:
CAN OPTIC FLOW FURTHER STIMULATE TREADMILL-ELICITED STEPPING IN NEWBORNS?
5.1 Abstract

Typically-developing three-day-old newborns take significantly more forward steps on a moving treadmill belt than on a static belt. The current experiment examined whether the addition of optic flows simulating forward translation would enhance forward stepping on a treadmill. Twenty-four newborns were supported on a moving treadmill without optic flow (no OF), with optic flow matching the treadmill’s direction and speed (congruent), with optic flow in the same direction but at a faster speed (faster), and with optic flow moving at the same speed but in random directions (random). The results revealed no significant differences in the rate or coordination of forward treadmill steps taken in each condition. However, significantly fewer vertical flexion-extension cycles (pumps) were made in the faster than the random condition. Comparison with a previous study suggests that step rates were close to ceiling because of the friction of the treadmill belt, thus limiting the effects of optic flow.

Locomotion, neonatal, perceptual-motor development, treadmill, optic flow

5.2 Introduction

5.2.1 The relevance of training stepping

Treadmill training has proven to be a very effective way to promote the acquisition of independent walking in infants with disabilities. Following Thelen and colleagues’ pioneering work (e.g., Thelen, 1986b, Thelen and Ulrich, 1991), several authors have shown that treadmill training not only hastens the onset of independent walking in infants with motor delays, but it also improves the quality of each step (Bodkin et al., 2003, Ulrich et al., 2008, Ulrich et al., 2001, Wu et al., 2007, see Teulier et al. (2013) for a review). These findings have raised questions about the optimal time to initiate interventions to maximise their effectiveness, particularly in light of a prevailing consensus that interventions should be initiated as early as possible to take advantage of the maximum degree of plasticity in early movement patterns, such as newborn stepping (Teulier et al., 2015, Ulrich, 2010).

A recent study by Siekerman et al. (2015), suggests that treadmill-based locomotor interventions for infants at risk of developmental delay could start at birth. They reported that typically-developing three-day-old infants made significantly more forward steps on a moving treadmill belt than on a static belt. Moreover, the newborns showed a degree of
specificity in their response to the speed of the treadmill belt, as they made significantly more steps when the belt moved at 0.172 m \text{ sec}^{-1} compared to 0.234 m \text{ sec}^{-1}. Arousal also had an influence on stepping. The steps were of higher quality and were more coordinated when infants were crying. Knowing that treadmill interventions could potentially be started at birth, an obvious question arises about the optimal conditions to promote early stepping. Treadmill speed and the infant’s arousal level influence the amount and quality of stepping, but what other factors might enhance stepping?

5.2.2 Augmenting stepping on the treadmill: optic flow

One factor that might enhance treadmill stepping in the newborn period is the availability of optic flow that specifies how the infant is moving relative to the support surface. Barbu-Roth and colleagues have shown that three-day-olds will make significantly more steps in the air when held upright above an optical treadmill – a pattern of optic flow that moves beneath the feet and specifies backward or forward displacement (Barbu-Roth et al., 2013, Barbu-Roth et al., 2009). In addition, typically developing infants between two and 10 months of age have been reported to increase their stepping frequency when the treadmill belt on which they were stepping was covered with a black-and-white checkerboard pattern, generating congruent optic flow, compared to when the belt was black or white, minimizing optic flow (Moerchen and Saeed, 2012). A similar finding has been reported for seven to 10-month-old infants with myelomeningocele (Pantall et al., 2012, Pantall et al., 2011). Together, these findings suggest that it may be possible to enhance newborn treadmill stepping by exposing infants to optic flows that specify forward translation while they are on a treadmill belt that moves in the same direction. The current experiment sought to test this notion.

5.2.3 Aims

The experiment was an extension of the experiment reported by Siekerman et al. (2015). Newborns were supported on a moving treadmill without additional optic flow, or with optic flows that moved in the same direction and at the same speed as the treadmill belt or at a faster speed, or were moving at the same speed as the treadmill belt but in random directions. It was hypothesised that step frequency and step quality would be enhanced by the optic flows that moved in the same direction as the treadmill belt, but not by the random optic flows. If visual input exerted a more powerful influence on stepping than the tactile/mechanical input from the treadmill belt, step frequency was expected to be
higher and with an improved quality in the faster optic flow condition compared to the congruent optic flow. Finally, consistent with the findings reported by Siekerman et al. (2015), it was predicted that step frequency and quality would be higher when newborns were crying.

5.3 Methods

5.3.1 Participants and Ethics
Twenty-four newborns were recruited from the maternity ward of the Port Royal Maternity Hospital in Paris. Only healthy infants born with an uncomplicated delivery, a minimum term of 38 weeks, an Apgar score of 10 at the fifth minute and a minimum birth weight of 2500 grams were selected. Four newborns were excluded from the final sample because these infants fell asleep during the experiment (see Chapter 3.1 for more details). The final sample included 20 newborns (12 m, 8 f; birth weight 3367 (±482.8) grams; age 2.7 (±0.7) days; mean term 40.1 (±0.9) weeks). Ethical approval was obtained from the ethics medical board of Paris-Ile de France and parents and caregivers gave informed consent for infant participation.

5.3.2 Experimental set-up and materials
An infant sized treadmill (belt surface 0.31 m x 0.59 m) was recessed into a 1 m x 1 m x 2 m (h x w x l) table so that both surfaces were level. The treadmill belt was covered with a white Dycem film (Dycem Ltd., Bristol, United Kingdom) to match the color of the table surface so that the surface appeared to be continuous. Optic flow was created with a MATLAB programme, run on a desk top computer and projected distortion-free onto the table and treadmill surfaces with a Sanyo PLC-XL51 projector (Sanyo Electric Co., Ltd., Osaka, Japan). The visual stimuli consisted of black, 0.045 m diameter dots on a white surface in the experimental conditions, with a black-white distribution of 37% black and 63% white (combined luminance 36.78 cd/m^2, for a visualisation, see Figure 17b). The dots were arbitrarily distributed across the optic flow surface and overlapped at random. The no optic flow condition (no OF) consisted of a neutral, static grey projection (luminance 14.97 cd/m^2, see Figure 17a). Four different visual stimuli were presented together with the treadmill moving at 0.134 m • sec\(^{-1}\). In the no OF condition, treadmill stimulation was combined with the grey projection. For the optic flow conditions, the dots appeared on one edge of the display.
surface and translated across to fall off the opposite edge. In the random condition, the
dots moved in random directions at 0.134 m • sec⁻¹ (24 deg • sec⁻¹), overlapping when
their paths crossed. In the congruent condition, the dots moved at the same speed and in
the same direction as the treadmill belt to mimic forward motion. In the faster condition,
the dots moved in the same direction but slightly faster (0.172 m • sec⁻¹ or 29 deg • sec⁻¹)
than the treadmill belt. The speeds of the optic flow and the treadmill were selected based
on the previous experiment by Siekerman et al. (2015). Optic flow speeds were slightly
faster than utilised by Barbu Roth due to the position of the newborns (Barbu-Roth et al.,
2013, Barbu-Roth et al., 2009), but comparable to Jouen’s maximal optic flow speed
(Jouen et al., 2000). The treadmill velocity was not at the optimal velocity of 0.172 m •
sec⁻¹ because of the need to leave room for optic flow to have an effect.

Figure 17: Visualisation of the optic flow conditions: a) no optic flow and b) optic flow
(random, congruent and faster)

Two high definition Sony CHDR-CX160 cameras (Sony Corporation, Tokyo, Japan)
were placed at 1.22 m height, to the left and right and 0.85 m away from the centre of the
treadmill, capturing movements at 60 Hz. An additional digital mini DV camera (Sony DCR-HC26) was placed at the front left of the infant (30 Hz).

5.3.3 Procedure
Infants were tested after feeding, when they were alert and not crying. They were dressed in a black sleeveless undergarment, and supported under the armpits by the experimenter’s hands (upright and angled slightly forwards, with the head and neck supported by the thumbs and the index finger). The infants’ feet were in contact with the treadmill belt. The four different conditions were presented in random order in four one-minute trials. Infants were repositioned at the front end of the treadmill if they had not responded to the treadmill for more than 10 seconds, to allow the hip to move through flexion and extension.

If infants showed signs of high distress, e.g., desperate crying without pauses, or if the parents indicated their infant was too stressed to continue, they were removed from the treadmill before the end of the trial. As a result, trial times ranged between 34 and 58 seconds (see Appendix B for a full list of final trial times). Missing trials were not repeated because the newborns were only in an optimal condition for testing for a brief period of time. Note that due to the amount of care needed when testing newborns, complete blinding of the experimenter was impossible.

5.3.4 Data reduction
Video data were analysed frame-by-frame in SiliconCoach (SiliconCoach Ltd, Dunedin, New Zealand, version 7-0-2-2). Periods with excessive tester movement (e.g., during repositioning of the infant) or touches by a parent (e.g., to encourage or calm the infant) were excluded from the analysis because they led to movements that were not generated by the infant (see Appendix B for a full list of final trial times).

Movement coding: Movement categories were based on an earlier experiment on neonatal treadmill stepping (Siekerman et al., 2015) with two main movements: pumps and steps. Pumps included vertical flexion and extension cycles, regardless of whether the foot was in contact with the belt at the start or end of the movement. Steps displayed locomotive potential, with the foot travelling forward for more than half a foot length during swing. In the step quality category, steps were further divided into typical steps (large range of motion and clear swing and stance phases) and atypical steps (small range of motion or an interrupted, shuffling stance phase). For step coordination, unilateral steps from one
leg were labelled as single steps; simultaneous steps with two legs as parallel steps; steps that were followed by a step from the opposite leg within 20-80% of its step cycle as alternated steps; and steps that were initiated between 80% of the opposite step cycle and one second after the finish of that step cycle as serial steps. Temporal characteristics, including step cycle duration and stance ratio (the time the foot was on the surface divided by the step cycle time) were determined under the cycle analysis.

_Arousal coding:_ Arousal was coded using a combination of all the video footage (from the left, right and left-front), depending on the direction of the newborn’s face. The scheme used to code arousal was identical to that used in the previous study (Siekerman _et al._, 2015) and based on Thelen’s six-point scale adaptation (Thelen _et al._, 1984) of Prechtl’s behavioural states (Prechtl, 1974). Arousal was sampled at five second consecutive intervals, recording 1) gross body movement, 2) if eyes were open or closed and 3) level of vocalisation. Precise mapping between stepping and arousal made it possible to examine whether the stepping parameters changed between less aroused, non-crying states (state 4) and highly aroused, crying states (state 5 and 6). This was done by assigning a state code to each step and pump, based on the arousal level of the five second time window in which the majority of the movement occurred.

_Looking time:_ Looking time was derived from the arousal coding. As active looking is not strictly necessary for optic flow perception, looking time was based on the time that the newborn’s eyes were open.

### 5.3.5 Statistical analysis

Nonparametric tests were used (Friedman tests and Signed-rank Wilcoxon tests for between-subject effects and for the arousal analysis) because most parameters were not normally distributed. The distributions were normal for the analysis of the effects of condition or trial order on arousal level, therefore parametric repeated measures analyses of variance (RM ANOVAs) were used. For more information on the statistical methods and reporting style, see Chapter 3.

### 5.4 Results

#### 5.4.1 Eye opening time

Newborns had the eyes open for an average of 20.3 seconds (± 22.4 seconds) per 60 second trial (for a full overview, see Appendix B), and a RM ANOVA showed no
significant different across conditions ($F_{3, 51} = 0.374; p = 0.772$). Because eye opening time was not equally distributed across conditions and quite variable across subjects, an additional exploratory analysis was performed on a sub-sample of infants in which eye opening time was more than 30 seconds per trial (n = 6). Additionally, this sub-analysis was restricted to the time in which the eyes were open. This analysis showed no significant differences across conditions or arousal states for step and pump rate, quality, coordination and cycle analysis, therefore the main results include the data from the entire sample (n=20).

5.4.2 Combined optic flow and treadmill effects on stepping

The main results are presented in Table 4. No significant differences were found in the step quality category (typical steps, atypical steps and typical step percentage) and the cycle analysis (cycle duration and stance ratio). These categories are therefore omitted from this results section.

Table 4: Step parameters by optic flow condition, with descriptive and inferential statistics for the parameters measured in the no OF, random, congruent and faster conditions. Note that the percentages do not sum to 100%, since medians and not means are reported.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>χ2</th>
<th>N</th>
<th>P-value</th>
<th>No OF</th>
<th>Random</th>
<th>Congruent</th>
<th>Faster</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Movement type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step (sec⁻¹)</td>
<td>1.320</td>
<td>20</td>
<td>0.724</td>
<td>0.411</td>
<td>0.310</td>
<td>0.397</td>
<td>0.248</td>
</tr>
<tr>
<td>Pump (sec⁻¹)</td>
<td>12.076</td>
<td>20</td>
<td>0.007</td>
<td>0.096</td>
<td>0.108</td>
<td>0.082</td>
<td>0.048</td>
</tr>
<tr>
<td>Step (%)</td>
<td>7.045</td>
<td>20</td>
<td>0.070</td>
<td>78.08</td>
<td>73.62</td>
<td>84.52</td>
<td>85.71</td>
</tr>
<tr>
<td>Single steps (%)</td>
<td>3.523</td>
<td>20</td>
<td>0.318</td>
<td>55.34</td>
<td>59.85</td>
<td>51.49</td>
<td>53.94</td>
</tr>
<tr>
<td>Parallel steps (%)</td>
<td>0.397</td>
<td>20</td>
<td>0.941</td>
<td>22.94</td>
<td>22.50</td>
<td>21.11</td>
<td>19.72</td>
</tr>
<tr>
<td>Alternated steps (%)</td>
<td>1.401</td>
<td>20</td>
<td>0.705</td>
<td>15.23</td>
<td>7.92</td>
<td>3.85</td>
<td>4.84</td>
</tr>
<tr>
<td>Serial steps (%)</td>
<td>0.509</td>
<td>20</td>
<td>0.917</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Movement type**

The median number of steps per second ranged from 0.248 steps per second in the faster condition to 0.411 steps per second in the no OF condition (Table 4). A Friedman test showed no significant differences between conditions. However, a significant difference was found for pumps per second ($\chi^2 = 12.076, p = 0.007$): post hoc tests (Wilcoxon Signed-rank) showed that in the random condition, more pumps per second (median 0.108 pumps per second) were made than in the faster condition (median 0.048 pumps per second).
per second; $Z = -2.240, p = 0.025$), however, after Bonferroni-Holm correction, this difference was not significant (Figure 18). No significant differences were observed for the percentage of steps across conditions (Figure 19).

**Step coordination**

For coordination, no significant differences were found between the different conditions. Single step percentage ranged from median 51.49% in the congruent condition to median
59.85% in the random condition, and parallel step percentage ranged from median 19.72% in the faster condition to median 22.94% in the No OF condition (Figure 20a). Alternated step percentage ranged from median 3.85% to median 15.23%, and the medians for serial step percentage were 0% in all conditions (see Figure 20b).

Figure 20: The effects of optic flow condition on step coordination, with a) single and parallel step percentage and b) alternated and serial step percentage.
5.4.3 Arousal effects on stepping

Arousal was not affected by condition or trial order. A RM ANOVA showed no increase for arousal in any of the conditions. Mean arousal level ranged from 3.96 in the random condition to 4.12 in the faster condition ($F_{3, 51} = 0.298, p = 0.827$). Additionally, mean arousal level did not increase for each consecutive trial. Means ranged from 3.94 in trial 3 to 4.19 in trial 1 ($F_{3, 51} = 0.467, p = 0.706$). Friedman tests showed no systematic increase or decrease in the number of movements per second across trials, with the higher number of movements made in the first (median 0.488 movements per second) and last trial (median 0.479 movements per second) and the least movements made in the third trial (median 0.404 movements per second; $\chi^2 = 7.320, p = 0.062$). Stepping also remained stable across the consecutive trials ($\chi^2 = 1.140, p = 0.767$). The effects of arousal on movement type and step coordination described in Table 5.

Table 5: Step parameters by arousal state, with descriptive and inferential statistics for the parameters measured when infants were crying or not crying. Note that the percentages do not sum to 100%, since medians and not means are reported. The analyses in some categories contain smaller sample sizes due to incomplete data sets that were excluded in the nonparametric statistical tests; some infants did not move or step in each category.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Z-value</th>
<th>N</th>
<th>P-value</th>
<th>Crying</th>
<th>Non-crying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Movement type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step (sec⁻¹)</td>
<td>-1.161</td>
<td>14</td>
<td>0.245</td>
<td>0.439</td>
<td>0.370</td>
</tr>
<tr>
<td>Pump (sec⁻¹)</td>
<td>-2.229</td>
<td>14</td>
<td>0.026</td>
<td>0.163</td>
<td>0.093</td>
</tr>
<tr>
<td>Step (%)</td>
<td>-1.852</td>
<td>14</td>
<td>0.064</td>
<td>71.01</td>
<td>80.54</td>
</tr>
<tr>
<td>Single steps (%)</td>
<td>-2.919</td>
<td>14</td>
<td>0.004</td>
<td>39.77</td>
<td>61.88</td>
</tr>
<tr>
<td>Parallel steps (%)</td>
<td>-2.261</td>
<td>14</td>
<td>0.024</td>
<td>35.42</td>
<td>15.67</td>
</tr>
<tr>
<td>Alternated steps (%)</td>
<td>-1.538</td>
<td>14</td>
<td>0.124</td>
<td>22.02</td>
<td>13.58</td>
</tr>
<tr>
<td>Serial steps (%)</td>
<td>-1.503</td>
<td>14</td>
<td>0.133</td>
<td>2.38</td>
<td>3.73</td>
</tr>
</tbody>
</table>

Movement type

Newborns did not produce a significantly different number of steps per second when they were crying compared to when they were not crying. However, the number of pumps per second was higher when newborns cried (median 0.163 pumps per second) compared to when they were in alert, non-crying states (median 0.093 pumps per second; $Z = -2.229$, $p = 0.026$, see Figure 21). No significant step percentage differences were observed between the different arousal levels (Figure 22).
Step coordination was affected by arousal: Figure 23a shows that when the newborns were crying relative to not crying, they had a significantly smaller percentage of single steps (39.77% compared to 61.88%, respectively; \( Z = -2.919, p = 0.004 \)) but a significantly higher percentage of parallel steps (35.42% compared to 15.67%,...
respectively; $Z = -2.261, p = 0.024$). There were no significant differences for alternated steps or serial steps as a function of crying (Figure 23b).

Figure 23: The effects of arousal level on step coordination with a) single and parallel step percentage and b) alternated and serial step percentage
5.5 Discussion

The absence of improvement in step count, quality, or coordination when optic flow was added to the treadmill failed to confirm the expectation that forward motion simulating optic flows added to a moving treadmill would enhance newborn stepping. Similarly, no evidence was found to support the hypothesis that the faster optic flow would enhance stepping relative to the optic flow moving at the same rate as the treadmill belt. The effects of arousal on stepping also did not conform to expectations. More pumps were seen when newborns were crying, but not more steps. In addition, more parallel steps were seen when infants were crying but not more alternating steps. Two factors potentially explain the pattern of findings, 1) the treadmill dominated the amount and quality of stepping because of the rudimentary nature of the coupling between vision and stepping, and 2) the step counts were already at their optimal level because of the Dycem material used to cover the treadmill belt.

5.5.1 The rudimentary coupling between vision and stepping

Despite showing that optic flow enhanced air-stepping in newborns, Barbu-Roth and colleagues (2013, 2009) have argued that the coupling between vision and stepping is rudimentary, at best. The coupling actually seems to weaken over the first couple of months of life (Anderson et al., 2016, Barbu-Roth et al., 2015), much like the stepping pattern itself when tested on a static surface, only to strengthen as the infant gains experience using vision to control movement. This idea is consistent with prior work showing that optic flow on a treadmill belt is more likely to facilitate stepping in older infants than younger infants (Pantall et al., 2012, Pantall et al., 2011). Thus, the combination of optic flow on a moving treadmill may simply not have been effective yet at birth.

Why was a visuo-locomotor link not observed in this experiment? Improvements in the visual control of locomotion appear to parallel improvements in the visual control of posture (Anderson et al., 2004), both of which are modulated by improvements in the ability to differentiate patterns of optic flow (Gilmore et al., 2004). The visual control of posture and locomotion may take longer to develop than somatosensory-based or vestibular-based control. For example, seated four-month-old infants displayed poorly organized muscular responses in the neck and trunk when they were rapidly translated forward and backward, but showed much more appropriate responses when they were deprived of vision by opaque goggles (Woollacott et al., 1987). Additionally, infants with
locomotor experience were more attuned to the appropriate portions of the visual field (Higgins et al., 1996); to control their posture in a moving room, they responded to motion from the side-wall alone whereas pre-locomotor infants did not make postural adjustments in response to peripheral optic flow. They proposed that optic flow sensitivity develops as infants are exposed to meaningful optic flows while they self-locomote. It is quite likely that optic flow sensitivity for locomotion is not developed enough to be utilised on the treadmill at birth.

A poorly developed ability to integrate multiple sources of information may also have compromised the newborns’ ability to utilize optic flow to increase stepping. It was expected that the newborns could integrate the tactile input from the treadmill with the visual input from the optic flow to develop a coherent percept of forward motion across the surface. These expectations may have been inappropriate given that Corbetta & Snapp-Childs (2009) reported that infants as old as six to nine months had problems utilizing vision and touch simultaneously to plan hand and finger movements in a grasping task. They claimed that infants ‘cannot quite parse the sensory information diligently to adjust their response pattern, and therefore continue to respond on the basis of their motor tendencies’ (p. 57). If infants older than six months had problems using two sources of information simultaneously, then it is highly probable that newborns would have similar, and likely more severe problems. It is quite possible that the infants in the optic flow conditions experienced a sensory overload that interfered with their ability to make stepping movements on the treadmill.

5.5.2 Stepping rates were at their maximum level

It is also possible that the visuo-locomotor link could not be observed in this experiment because of a ceiling effect. A comparison of the step rate reported by Siekerman et al. (2015) when the treadmill belt moved at 0.134 m • sec⁻¹ compared to the step rate in the current experiment in the equivalent condition revealed that the rate was over double in the current experiment (0.41 vs. 0.19 steps•s⁻¹). Possibly, this difference was an unintentional consequence of the Dycem that covered the treadmill belt. Dycem is a high friction polymer used in therapeutic settings to reduce slipperiness of surfaces. Though the Dycem was originally used to ensure that the treadmill belt was the same white color as the surrounding table surface, it is important to note that previous experiments have shown that Dycem increases friction on the treadmill belt and has been shown to facilitate the rate of stepping in older infants (Pantall et al., 2012, Pantall et al., 2011). The Dycem
also increased the duration of the stance phase between the two experiments, perhaps explaining why there were no effects of condition on the cycle duration or stance ratio in the current experiment. Most importantly, the Dycem probably increased the stepping rates to ceiling levels across all conditions because it helped to ensure that stepping was primarily driven by the mechanical pull of the treadmill belt.

Is the strong Dycem effect beneficial in a training setting? To confirm this, further study needs to examine whether the Dycem was the only factor responsible for increasing step rate and quality so strongly. However, if Dycem could maximise stepping at birth, it may be very useful, particularly for infants with a low response rate due to disabilities or motor delay. Augmented step training will reinforce neural patterns within the stepping movement where these experiences would normally not be gained. It should be noted that treadmill stepping, Dycem-augmented or on a smooth belt, may be regulated more strongly by neural structures in the spinal cord, whereas spontaneous movement - and perhaps air-stepping with optic flow – could be mainly controlled at higher neural levels. Tactile-proprioceptive reinforcement may emphasise spinal pathways over cortico-spinal pathways, and may therefore have different effects on groups with different disabilities. Indeed, Dycem may be useful in some populations, whereas another stimulus, such as optic flow, may support the development of weak pathways in others.

The Dycem might also explain the selective nature of the effects of arousal on leg movements. High arousal (crying) had no effect on step counts but significantly increased the number of pumps. Because the pumps are primarily vertical movements that involve limited contact between the foot and the treadmill surface, this category of leg movement was perhaps the only one that was open to modification by some factor other than the treadmill belt. Similarly, the Dycem might explain the effects of arousal on coordination. Whereas high arousal previously improved step alternation (Siekerman et al., 2015), no significant differences were observed for alternated steps in the current study. This may be because alternating steps were close to their maximum rate in the current experiment compared to the Sickerman et al. (2015) experiment (15% of steps vs. 0% of steps in the comparable 0.134 m • sec⁻¹ conditions). Parallel steps did increase when arousal was high, however, and single steps decreased. We propose that the tendency to tighten interlimb coordination when arousal increases – as suggested by Sickerman et al. (2015) - leads to an increase in alternating and parallel steps, but only led to a significant increase in parallel steps in the current experiment because alternating steps were already close to their maximum response level.
The fact that high arousal appears to improve movement rate at birth, even if it did not advance movement quality in this experiment, might be perceived as a useful factor for clinical applications. Particularly in newborns at risk of developmental delay, who may show lower response rates, any improvement of stepping may be desirable. However, it must be noted that crying – a distress signal - must have negative effects that may be greater than the possible benefits. Crying has multiple short and long term effects (Ludington-Hoe et al., 2002), with among the short-term effects an increase in cortisol levels and blood pressure, and a decrease in oxygenation levels. There are indications that early negative experiences (crying in response to pain or stress) affect behaviour at a later age (Taddio et al., 1997; Douglas and Hill, 2013). It seems prudent to attempt to improve stepping through alternative stimuli than high arousal.

5.6 Limitations

There are some limitations that need to be taken into account with the current experiment. The Dycem elicited an unexpectedly strong response, which in itself may be an indication of increased tactile/proprioceptive sensitivity in newborns. The lack of results from the different optic flow conditions may be due to a ceiling effect or due to a lack of newborn sensitivity - or a combination -, but in the current experimental set-up these possible explanations cannot be disentangled. This can only be rectified with a subsequent experiment where newborns are exposed to optic flow alone, a moving treadmill alone (with and without Dycem), and a combination of optic flow and a moving treadmill.

Additionally, the optic flows were at the upper range of the currently utilised optic flow speeds for newborns and may have been too high. Treadmill speed needed to be matched to optic flow, and if treadmill speed had been based on a middle range of optic flows, the treadmill speed would have been far below the optimum. As the research question aimed to investigate the additional benefits from optic flow on a moving treadmill, a range of effective treadmill speeds was chosen as the foundation speed. Additionally, the fastest optic flow speed was similar to the fastest optic flow speed in Jouen’s experiment (2000), which was able to elicit a response. It is, however, possible that the effects begin to diminish around 30 degrees per second. The fact that optic flow speeds were not optimal may have made newborns more likely to respond strongly to the stimulus of the (optimal) treadmill speed alone. Therefore, an experiment at sub-optimal treadmill speed - i.e., at optic flow speeds at which air-stepping could be elicited (Barbu-Roth et al., 2009) - may
be better able to determine the effect of optic flow on the treadmill – even if lower step rates are achieved. Furthermore, eye opening time, or the time that infants were able to perceive the optic flow, may have affected the results of the experiment. However, Jouen et al. (2000) successfully elicited postural responses in newborns exposed to different optic flows in trials of merely 10 seconds. Nonetheless, it is possible the link between optic flow and stepping requires a different length of exposure. Additionally, it was not entirely clear how much optic flow was perceived by newborns in this experiment, because it is very difficult to establish whether the eyes are open or not.

Lastly, it is essential to bear in mind that the primary aim of the experiment was to assess whether an optic flow added to a moving treadmill would augment the treadmill facility. It would have been possible to examine the effects of treadmill speeds and congruency in more detail by using different optic flow-treadmill speed combinations, but this fell outside of the scope of this experiment.

5.7 Conclusion

In summary, adding optic flow to a moving treadmill belt does not enhance the amount and quality of newborn forward stepping in the context in which stepping was elicited in the current experiment. It is possible that optic flow did not enhance treadmill stepping in newborns because of the rudimentary nature of the coupling between vision and stepping or the newborn’s limited capacity to simultaneously process more than one source of information. A more likely explanation is that forward stepping was at ceiling because of the Dycem on the treadmill belt. Consequently, optic flow would not have had any additional benefits. The Dycem may also explain the limited effects of arousal on stepping. Though unexpected, the effect of Dycem is a very important finding in its own right because it suggests that enhancing friction on the treadmill surface is a powerful way to enhance the effectiveness of very early treadmill stepping interventions. Further work is clearly warranted to understand how combinations of sensory input influence early stepping and how early locomotor interventions can be optimized.
Chapter 6:
A DETAILED KINEMATIC ANALYSIS OF STEPPING ON THE TREADMILL WITH ADDED OPTIC FLOW
6.1 Introduction

The importance of attaining independent walking at an appropriate age – with minimal delay – is emphasised by the strong link between upright locomotion and the boost in development in sensori-motor, cognitive and socio-emotional domains (Campos et al., 2000, Anderson et al., 2013) and changes in intra-familial relations (Biringen et al., 1995). Additionally, absence of self-locomotive experience is reflected in the delay in attainment of certain cognitive skills (Campos et al., 2009). It is therefore essential to give children at risk of developmental delay the opportunity to minimise delay of the onset of independent walking (Ulrich, 2010, Ulrich et al., 2001). Early and aggressive intervention is most effective (Ulrich, 2010), and the use of the infant’s ability to produce coordinated, step-like movements in treadmill training paradigms has been very successful at bringing forward walking onset and improving kinematics in infants with Down syndrome (Ulrich et al., 2001, Wu et al., 2007, Ulrich et al., 2008, Wu et al., 2010).

Upright, tactile stepping is observed from birth and, although kinematically somewhat different, has been placed in a developmental continuum with voluntary supported infant stepping and independent locomotion (Thelen and Cooke, 1987, Dominici et al., 2011). It is now thought that stepping at birth is expressed in other contexts, including in the air (Barbu-Roth et al., 2009), on a moving surface (Siekerman et al., 2015) and in prone crawling (Forma et al., 2015). Stepping can be trained from a very early age (Zelazo et al., 1972, André-Thomas and Autgaerden, 1966), although training interventions are typically initiated when infants are able to sit (Ulrich et al., 2001). A recent study suggested that interventions could be initiated as early as directly after birth (Siekerman et al., 2015). However, stepping at birth is not always predictably evoked, and tends to attenuate further from about two months of age when not trained (McGraw, 1940, Yang et al., 1998b). Therefore, it is important to investigate ways to further augment training paradigms and determine what stimulates stepping optimally.

6.1.1 Optic flow to elicit stepping

Previous experiments have shown that upright stepping (or its supine kicking counterpart) can be influenced by sensory stimulation (Thelen et al., 1984, Thelen, 1994). Evidence for the effects of a visual treadmill was already found for three day old infants who showed responses to optic flow projected on the surface beneath their feet when they were supported in the air; Barbu-Roth and colleagues found that newborns air-stepped more in response to optic flow simulating forward movement compared to a static or rotating
high-contrast surface (2013, 2009). The visual stimulus elicited stepping at rates similar to those elicited by the traditional static tactile stimulus. Moreover, when tested upright with their feet in contact with a silicone mattress (Barbu-Roth et al., 2016) or in a prone quadrupedal position on a transparent water mattress (Forma et al., 2015), three day old newborns significantly increased tactile leg step rate in response to optic flows on the surface below the mattress. Thus, optic flow and tactile stimuli may be able to enhance each other as early as directly after birth. However, Chapter 5 showed that in three-day-old newborns, optic flow on the treadmill did not increase step rate or modify coordination. Conversely, step frequency (Pantall et al., 2011) and muscle activation (Pantall et al., 2012) was enhanced in infants with spina bifida (aged two to 10 months) who stepped on a moving treadmill belt enhanced with optic flow, although the effect was stronger in the older group. In infants with typical development of the same age group, step rate responded similarly, but there was no age effect (Moerchen and Saeed, 2012). Perhaps infants need at least some visual experience to respond to optic flow in combination of dynamic tactile-propiroceptive feedback. Additionally, infants from two months of age may be better able to process multi-modal information than newborns (Anderson et al., 2004, Anderson et al., 2001). However, it is also possible that at birth, the tactile stimulus overrode the effects that the optic flow might have produced (Chapter 5). This may be especially true because the treadmill had been augmented with additional proprioceptive elements (Dycem). Nevertheless, optic flow may have had more subtle effects that were only observable at deeper levels of analysis than step count. Barbu-Roth et al. (2013, 2015) showed that optic flow direction can affect joint kinematics, and Pantall et al. (2012) saw clear effects for muscle activity in response to optic flow. Even though these changes were accompanied by step count increases, it is still possible that changes in newborns occur only within joint kinematics or neuromuscular behaviour.

6.1.2 The proprioceptive and tactile stimulus
It should be noted that the strength of the tactile stimulus at birth is well-known. Placing a newborn infant on a solid surface is the classic method to elicit stepping (Domellöf et al., 2007, Dominici et al., 2011, Yang et al., 1998a, Thelen and Fisher, 1982). The introduction of treadmill movement underfoot appears to enhance static tactile stimulation (Siekerman et al., 2015). This did not only affect movement rate, but the
quality of the movement also shifted from mainly vertical flexion-extension cycles to real ‘steps’ resembling terrestrial walking.

The treadmill had also been identified by Thelen and colleagues as able to shift movement patterns towards a more ‘mature’ expression (Thelen, 1986b) in seven-month-old infants. Although they had lost the ability to step in response of the classic stimulus - i.e., upright in a gravity environment and on a static surface - they regained their stepping pattern on the treadmill, showing more advanced, alternating interlimb coordination with increased decoupling between joints. The treadmill provided a whole realm of new tactile-propiocceptive stimuli to explore. Ulrich experimented with the modality of the treadmill and discovered that the treadmill surface texture mattered; infants with Down syndrome responded best to wearing Velcro socks on a Velcro surface (Ulrich et al., 1998). Friction, through the addition of Dycem to the treadmill belt, also improved stepping in infants with spina bifida (Pantall et al., 2011). The treadmill’s efficacy was attributed to the added energy from the treadmill, which allowed infants to overcome leg inertia (Thelen, 1986b), or assisted them in reaching sufficient hip extension, which is thought to trigger (Pang and Yang, 2000) or mechanically facilitate further stepping (Thelen and Cooke, 1987). Additional to providing a very strong proprioceptive-tactile stimulus, treadmills also make the delivery of the stimulus easier and more controllable, which has made it particularly useful in training paradigms (Ulrich et al., 2001). However, while the effect of the treadmill on older infants is probably a combination of passive and active stimulation of the legs joints and muscles, it is very well possible that it is more passive in newborns, especially at the velocities tested in our experiments. This is perhaps why no improvement in step rate with speed, or a switch from pumps to steps, was observed in the previous chapter: it may have been difficult for these newborns to overcome the passive drive of the treadmill to change their step rate and interlimb coupling in response to added optic flow, especially if the tactile stimulus was further augmented with Dycem.

6.1.3 Arousal facilitates movement

The facilitative effects of sensory stimulation - tactile-propioceptive and visual - are well-reported in newborns. However, Thelen et al. noticed that in newborns (age two to six weeks of age) arousal best predicted stepping (1984). In terms of general movements, Hadders-Algra and colleagues (1993) showed that infants moved more vigorously, and with larger muscle contractions, when they were crying (Prechtl’s state 5, Prechtl, 1974). Siekerman et al. (2015) showed that arousal can also affect stepping; infants stepped more
when they were crying (state 5 and 6 - comparable to Prechtl’s state 5) compared to when they were not crying but moving and alert (state 4). However, the study also found an effect for step quality and coordination. Smoother, further forward placing steps with tighter alternation were observed during higher arousal, compared to the sometimes shuffling, smaller steps that were seen mostly when newborns were less aroused. This led to the proposal that arousal may also shift the control of stepping, making the steps not only larger, but also more automated or reflex-like. There is some evidence that arousal affects the automaticity of movement patterns. McEwen et al. (1997) reported that ‘L-dopa-induced free swimming was more stereotyped and ‘automatonlike’ than that of vehicle-injected animals’. Pattern generation in spinalised animal preparations is typically facilitated by pharmaceutical intervention, particularly through modulation of the noradrenergic and serotonergic systems (Rossignol, 1996a). However, there is little information on how these systems support locomotion in intact human infants in in-vivo contexts. There are no in-depth studies reporting either the effect of arousal on treadmill stepping at birth in terms of kinematics or muscle behaviour.

6.1.4 Aims
Changes in motor behaviour can be difficult to distinguish, as they may not be expressed through end-point performance. Although no explicit modifications to step rate and coordination were observed in the previous chapter, adaptation may have occurred at different control levels. Therefore, this experiment will further analyse the effects of sensory stimulation from the data described in Chapter 5. The focus in this chapter will be on changes to quantifiable kinematic parameters such as joint angles, and speed and range of motion. We expect that joint kinematics may be subtly affected by optic flow, but that tactile-proprioceptive treadmill stimulation is powerful and tends to mask newborns’ sensitivity to visual stimuli. Additionally, the question raised in Siekerman et al. (2015) is further explored: does arousal engender automaticity in stepping? Stereotypies were examined by investigating the march-like character of newborn stepping, characterised by high knee-hip synchronisation, reduced progression (step length) and improved movement smoothness (movement units). Arousal was predicted to synchronise hip and knee movement, reduce step length and decrease the number of movement units.
6.2 Methods

6.2.1 Participants and Ethics
Twenty-four newborns were recruited from the maternity ward of the Port Royal Maternity Hospital in Paris. For further details of the selection procedure, participant data and analysis inclusion criteria, see the method section of Chapter 5.

6.2.2 Experimental set-up and materials
The experimental set-up contained an infant-sized motorised treadmill that was recessed in a large table with both surfaces level. Both surfaces were white; the treadmill was covered with a white Dycem film (Dycem Ltd., Bristol, United Kingdom), so that optic flow could be projected on one continuous surface. Optic flow consisted of black and white dots moving in random directions but matching treadmill speed; congruent with the treadmill speed and direction (running at 0.134 m•s\(^{-1}\)); or faster (at 0.172 m•s\(^{-1}\)) than the treadmill but in the same direction. Two video cameras (60 Hz) were placed perpendicularly on the left and the right, and one camera was placed at the front and left of the infant (30 Hz). For more detail on the set-up, cameras and optic flow specifics, see the experimental set-up paragraph in Chapter 5.

6.2.2.1 Three-dimensional motion capture
An eight-camera Oqus 100 three-dimensional (3D) motion analysis system (Qualisys AB, Gothenburg, Sweden) was set up to capture kinematic data at a sampling frequency of 60 Hz. Newborns were dressed in a black sleeveless undergarment and retro-reflective markers (0.5 cm diameter) were placed, bilaterally, on the torso and the extremities. Markers incorporated in the infant suit (1 cm diameter) were located bilaterally at the acromion process, on the greater trochanter and in the centre of the neck, at the seventh vertebra. Additional retro-reflective markers (0.5 cm diameter) were placed on the left and right lateral wrist and elbow, knee and ankle (see Figure 24). One cm markers were also attached to the tester’s forearm, halfway between the wrist and the elbow. The system was calibrated at the start of each testing day, or if any of the cameras were accidentally touched. The system was synchronized between the 3D motion capture system and the high definition cameras as well as an additional EMG capture system (reported in Chapter
7). The frontal video camera could not be synchronized as all the available ports were utilised; these were later manually synchronised.

![Front view and Rear view of a figure showing marker placement](image)

**Figure 24: Marker placement**

<table>
<thead>
<tr>
<th>Front view</th>
<th>Rear view</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Image of marker placement" /></td>
<td><img src="image" alt="Image of marker placement" /></td>
</tr>
</tbody>
</table>

1. Neck marker (Cervical Spinous Process C7)
2. Shoulder markers (Acromion Process – left and right)
3. Elbow markers (Lateral Epicondyle of the Humerus – left and right)
4. Wrist markers (Ulnar Styloid Process – left and right)
5. Hip markers (Greater Trochanter – left and right)
6. Knee markers (Lateral Epicondyle of the Femur – left and right)
7. Ankle markers (Lateral Malleolus – left and right)

**6.2.3 Procedure**

Infants were tested when they were alert and not crying, by supporting them under the armpits with their feet in contact with the treadmill. The four one-minute conditions were presented at random and with approximately one minute rest in between the trials. Infants were removed if the experimenters or parents felt that infants showed excessive distress. Missing trials were not repeated, and complete blinding of the experimenter was impossible. For more details, see the procedure paragraph in Chapter 5.

**6.2.4 Data reduction**

Video data were analysed frame-by-frame in SiliconCoach (SiliconCoach Ltd, Dunedin, New Zealand, version 7-0-2-2). Coding parameters focused on two main movements: vertical pumps and forward steps (for a detailed description, see Chapter 3 and Appendix
A). Time stamps of steps and pumps were recorded (start of movement, touch-down and end of movement). Arousal was coded using a combination of all the video footage (from the left, right and left-front), depending on the direction of the newborn’s face. The scheme used to code arousal was identical to that used in the previous study (Siekerman et al., 2015); see Chapter 3 for a more detailed description.

6.2.4.1 Kinematic post-processing

3D motion analysis data were analysed entirely in Qualisys Track Manager (QTM, version 2.7, Qualisys, Gotheburg, Sweden). A six-degrees-of-freedom (6DOF; degrees of freedom include the x, y and z axes, and rotation about each of the respective axes) body model was created based on the markers used in the experiment (see Figure 25). A set length of 5 mm error was allowed for bone length, and automatic gap filling (polynomial interpolation) was set to a maximum of 10 frames. After application of the model, each trial was verified for marker tracking. Because of the infants’ position during steps, ankle markers were often masked, leading to many large gaps in the data and poor tracking. To maximise the amount of tracked data for analysis, gaps larger than 10 frames but smaller than 36 frames (0.16s-0.5s) were also filled by polynomial interpolation, but for these gaps, the movement of the marker was also visually examined by comparing marker movement to the 2D video to ensure it looked natural and resembled the infant’s movement. Due to a drastic reduction of subjects, exclusion of the entire subject in case a marker was missing for more than 50% of the time was not feasible. Friedman analyses on attrition showed that there was no difference in percentage marker loss across the conditions (ranging from median 30% to 37%, $\chi^2 = 1.171, p = 0.760$) or according to trial order ($\chi^2 = 2.471, p = 0.481$). Wilcoxon Signed-rank tests showed that there was no difference for marker loss between the left and the right leg ($Z = -1.328, p = 0.184$) or crying and non-crying steps ($Z = -0.804, p = 0.422$). Because marker loss was deemed to occur at random, all trials were included in the analyses, but steps and pumps in which one of the markers was missing were excluded.

Because QTM did not offer Butterworth filtering, a 2nd degree polynomial curve was fitted to the data. This filter eliminated small errors, but left the main signal intact. Visual inspection determined that the optimal filter window length was 5 frames. Qualisys QTM software was then applied to calculate the three-dimensional locations (x, y and z) of the ankle markers within a Cartesian coordinate system. The continuous relative angles between shank and thigh (the knee angle) and between torso and thigh (the hip angle)
were calculated. These angles were exported to Excel; step and pump time stamps were further used to extract the kinematic parameters. The following parameters were examined: step and pump length, joint angles (hip and knee maximal and minimal angles - with joint range of motion (ROM) through the subtraction of the minimal angle from the maximal angle in each step), joint angular velocities (hip and knee maximal and minimal angular velocity), inter-joint coupling (timing of minimal and maximal joint angles and angular velocities) and movement units (accelerations and decelerations of the ankle marker throughout the movement).

Figure 25: Body model (6 DOF) using 13 markers. Note the two floating markers on the forearm of the tester.

6.2.4.2 Kinematic parameters

Step and pump length: For step and pump length, the total forward (horizontal) excursion of the ankle marker at touchdown was calculated by subtracting the x-component at toe-off from the x-component at touchdown. We focused on the total forward component only, as it shows the clearest expression of functional locomotive potential in the upright position – i.e., in the context of walking.

Joint angles and angular velocities for steps and pumps: The 3D relative angles were calculated for the knee and hip with QTM software. Continuous angles and velocities were calculated over the whole trial. An excel program then extracted minima and maxima from angle and velocity traces during all movements (steps and pumps). Minima
and maxima were also calculated for the swing and stance phases of the steps exclusively, as no stance period was determined for the pumps. Negative values signify flexion rate, whereas positive values signify extension rate.

**Inter-joint coupling for steps and pumps:** The coordination between joints was examined by calculating the discrete relative phase between the hip and the knee. Time of peak knee flexion was subtracted from time of peak hip flexion, normalised by dividing by cycle duration and then multiplied with 360 degrees (Hamill et al., 2000). Values close to zero and 360 degrees signify in-phase relationships – complete synchronisation-, whereas values close to 180 degrees indicate an out of phase relationship. The time lag between the fastest extension and flexion rates was also calculated to illustrate the newborns’ synchronisation of the time in which their hip and knee moved fastest. The time of peak knee flexion rate was subtracted from the time of peak hip flexion rate, normalised by cycle time and expressed as percentage lag. Values close to zero signify strong synchronisation, whereas values close to 100% and -100% signify greater decoupling across the step cycle.

**Movement units:** To show the smoothness of a movement, the number of movement units was calculated according to an algorithm based on Von Hofsten’s method (1991). A movement unit (MU) consists of the acceleration and deceleration of a motor end-point. To qualify as a MU, the velocity of the end point increased with at least 10mm • sec\(^{-1}\) from start to peak, and decreased with at least 10mm • sec\(^{-1}\) from peak to finish. This criterion serves to filter out movement units that are too small and may be caused by the vibration from the treadmill. No minimum acceleration criterion was stated, as this examination also aimed to include the slower MUs as well. The number of MUs per step and pump was calculated, with fewer MUs signifying smoother movement.

**6.2.5 Statistical analysis**

Twenty-seven variables (step, pump and overall movement length; angles, angular velocities and range of motion for the hip and knee; inter-joint coupling and movement units for steps and pumps) were tested for effects of optic flow condition and arousal level. Because the majority of the variables were not normally distributed, nonparametric tests were used. Friedman tests assessed the effects between the four repeated measures optic flow conditions, with post hoc Signed-rank Wilcoxon tests to determine between which conditions the differences occurred. The arousal analysis was also performed using Signed-rank Wilcoxon tests. Post hoc results were corrected for multiple comparisons.
with the Bonferroni-Holm method (see Chapter 3.3 for more information on the statistical methods and reporting style).

Note that the high number of analysis makes the chance of type I error considerable (see Chapter 3.4.5). Additionally, the low sample size in some variables increases type II error and makes certain analyses very low powered (see Chapter 3.4.6): it is possible that certain effects were missed. However, these kinematic analyses addressed the qualitative aspects of what a newborn step on the treadmill may look like rather analysing it quantitatively. Hence, the discussion focused more on the trends that appear frequently in newborns. However, further studies are required to acquire a more detailed picture of newborn treadmill step characteristics.

6.3 Results

6.3.1 Step and pump length
The main results are printed in Table 6. Individual profiles are displayed in Appendix C to reflect the high level of response variability (Figure 42 to 54). There were no significant differences in step or pump length for the different conditions (Figure 42). Step lengths ranged from a median of 34.3 mm in the random optic flow condition to 45.3 mm in the congruent condition, whereas pump lengths ranged from a median of 6.4 mm in the congruent condition to 23.4 mm in the faster condition. Even though these differences appeared substantial they were not significant. Note that because of attrition, sample sizes are diminished. Step and pump lengths were also not significant different when arousal level changed (Figure 42). The median for steps was 42.7 mm when newborns were crying compared to 36.1 when not crying. The difference for pumps appeared quite large with a median of 2.7 mm when newborns cried and 18.2 mm when they did not cry, although this difference was not statistically significant.

6.3.2 Joint angles
There was a significant difference for the peak hip extension as seen for the whole cycle. The congruent condition showed a significantly smaller hip angle (median 132.4 degrees) during maximal extension than the random condition (median 137.0 degrees; \( \chi^2 = 10.875, p = 0.012 \)). Differences between the no OF condition and the faster condition and the no
OF and the congruent condition failed to reach significance when adjusted with the Holm-Bonferroni correction.

There were no further significant differences for peak hip extensions (during stance), for peak hip flexions (during the whole cycle), nor were there significant differences for the knee angles when comparing different conditions. For an impression of individual responses, see Figures 43 and 44 in Appendix C.

When looking at changing arousal level, there were significant differences between crying and non-crying for peak extension during stance, and peak flexion and extension while considering the whole cycle. During stance, the hip was significantly more extended when crying (median 137.2 degrees, compared to median 130.3 degrees when not crying; $Z = -1.988, p = 0.047$). Correspondingly, the hip showed a more extension when crying during the entire cycle (median 137.7 degrees compared to median 128.0 degrees when not crying; $Z = -2.621, p = 0.009$). Conversely, the infants were less flexed (during peak flexion) while crying (median 110.4 degrees) compared to when they did not cry (median 109.8 degrees; $Z = -2.970, p = 0.003$). Overall, newborns showed more extended hip angles when they cried compared to when they did not cry (see Figures 45 and 46 in Appendix C).

### 6.3.3 Range of motion

There were no significant between-condition differences for the cycle ROM of the hip and the knee; minimal hip ROM occurred in the congruent condition and its maximum was reached in the random condition (median 17.8 and median 22.1 degrees respectively) whereas minimal knee ROM was observed in the random condition with its maximum occurring in the no OF condition (median 15.0 and median 21.0 degrees respectively). For the arousal state analysis, knee ROM was significantly smaller when newborns were not crying compared to when they cried (median 15.3 compared to median 20.3 degrees; $Z = -2.481, p = 0.013$). A similar, nonsignificant trend was found for hip ROM (median 17.6 compared to median 25.0 degrees). For an overview of individual responses, see Figure 47 in Appendix C.

### 6.3.4 Angular velocities

No significant differences were found for angular velocities across the different optic flow conditions. There were no significant changes for maximal hip or knee extension and flexion rates.
However, the angular velocities were clearly impacted when infants were crying. During the stance phase, maximum extension rate was higher, with a maximum angular hip velocity of median 60.9 deg • sec\(^{-1}\) when newborns cried compared to median 44.0 deg • sec\(^{-1}\) when they did not cry (Z = -2.701, p = 0.007). Correspondingly, there was a similar significant difference across the whole cycle with a peak extension rate of median 73.6 deg • sec\(^{-1}\) during crying compared to median 52.0 deg • sec\(^{-1}\) when newborns did not cry (Z = -2.201, p = 0.028). Newborns also flexed their knees most rapidly when crying, with a minimum angular velocity of median -114.8 deg • sec\(^{-1}\) when they were crying compared to median -71.1 deg • sec\(^{-1}\) when they did not cry (Z = -2.201, p = 0.028). For the remaining angular velocities, flexion and extension rate maxima tended to be consistently higher when newborns were crying, although these differences were not statistically significant (see Appendix C, Figures 48 to 51 for individual plots).

6.3.5 Inter-joint coupling

There were no significant differences for the discrete relative phase of peak knee and hip flexion across the four conditions. Similarly, there was no significant difference for relative phase of peak knee and hip extension. For the peak angular velocity lags, there was a significant difference for minimal angular velocity lag: post hoc comparisons showed that the timings of the minimal angular velocities of knee and hip (peak flexion rates) were significantly less synchronised in the congruent condition (median lag of 15.98%) than in the random condition (median lag of 4.29%; \(\chi^2 = 12.900, p = 0.005\)), with maximal hip flexion rate occurring before maximal knee flexion rate. There were no significant differences for the maximal angular velocity timings of knee and hip.

For different arousal levels, there was a significant difference for maximal angular velocity lag: peak extension rate of knee and hip was significantly more synchronised when crying, with a median lag of -2.30% compared to a median lag of -10.32% when they were not crying (Z = -2.589, p = 0.010), with the knee’s fastest extension rate occurring before the fastest hip extension rate. Additionally, for 13 out of 16 infants, lag increased between the random and congruent condition (see Figures 52 and 53 in Appendix C for individual profile plots). There were no further significant differences in this category.
Table 6: Kinematic parameters for steps and pumps between four conditions (no OF, random, congruent and faster) and between arousal (crying and non-crying) states. Note that minimal angles signify peak flexion and maximal angles peak extension. Similarly, minimal angular velocity signifies peak flexion rate (negative sign) and maximal angular velocity signifies peak extension rate (positive sign).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Medians</th>
<th>No OF</th>
<th>Random</th>
<th>Congruent</th>
<th>Faster</th>
<th>Crying</th>
<th>Non-crying</th>
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<tr>
<td>By Optic Flow Condition</td>
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<td></td>
<td></td>
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<td>Step and pump lengths</td>
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<tr>
<td>Step length</td>
<td>10</td>
<td>2.280</td>
<td>0.516</td>
<td>1131.49</td>
<td>208.365</td>
<td>109.206</td>
<td>106.557</td>
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<td>Pump length</td>
<td>7</td>
<td>2.314</td>
<td>0.510</td>
<td>6.849</td>
<td>1.3406</td>
<td>63.55</td>
<td>23.429</td>
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<td>Movement length²</td>
<td>13</td>
<td>2.354</td>
<td>0.502</td>
<td>24.019</td>
<td>27.020</td>
<td>25.32</td>
<td>34.212</td>
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<td>Joint angles</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hip Minimum angle (swing¹)</td>
<td>10</td>
<td>2.280</td>
<td>0.516</td>
<td>1131.49</td>
<td>208.365</td>
<td>109.206</td>
<td>106.557</td>
</tr>
<tr>
<td>Maximum angle (stance¹)</td>
<td>10</td>
<td>2.280</td>
<td>0.516</td>
<td>135.826</td>
<td>133.475</td>
<td>133.863</td>
<td>128.239</td>
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<tr>
<td>Minimum angle (whole cycle²)</td>
<td>16</td>
<td>3.225</td>
<td>0.358</td>
<td>114.282</td>
<td>115.009</td>
<td>111.093</td>
<td>110.527</td>
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<tr>
<td>Knee Minimum angle (swing¹)</td>
<td>10</td>
<td>4.680</td>
<td>0.197</td>
<td>105.966</td>
<td>104.131</td>
<td>109.022</td>
<td>110.451</td>
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<tr>
<td>Maximum angle (stance¹)</td>
<td>10</td>
<td>2.520</td>
<td>0.472</td>
<td>125.265</td>
<td>118.895</td>
<td>124.629</td>
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<td>109.913</td>
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<td>Joint ROM</td>
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<td>Hip range of motion (whole cycle²)</td>
<td>16</td>
<td>2.025</td>
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<td>Angular velocities</td>
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<tr>
<td>Hip Maximum angular velocity (stance¹)</td>
<td>10</td>
<td>5.640</td>
<td>0.131</td>
<td>57.070</td>
<td>50.19</td>
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<td>45.952</td>
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<td>Minimum angular velocity (swing¹)</td>
<td>10</td>
<td>0.480</td>
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<td>16</td>
<td>2.175</td>
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<td>Knee Maximum angular velocity (whole cycle²)</td>
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<td>16</td>
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<td>Angle (deg)</td>
<td>Lag (%)</td>
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<tr>
<td>Peak flexion (whole cycle)</td>
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<td>Peak extension (whole cycle)</td>
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<td>Angular velocity maximum</td>
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<td>Angular velocity minimum</td>
<td>12.900</td>
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<table>
<thead>
<tr>
<th>Movement units</th>
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<tr>
<td>MU step</td>
<td>0.943</td>
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<tr>
<td>MU pump</td>
<td>1.350</td>
<td>4.875</td>
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</tbody>
</table>

Note that negative relative phase indicates that the hip led the knee, and positive relative phase specifies that the knee led the hip.

For 1: steps alone were analysed and for 2: steps and pumps were analysed simultaneously.
6.3.6 Movement units
There were no significant differences in this category. Movement units during stepping at different optic flow conditions ranged from 7.6 units in the no OF condition to 8.0 units in the congruent condition. For pumps, the least movement units were found in the no OF condition compared to the random condition (4.7 and 7.5 units respectively). For the arousal analysis, less movement units during stepping were observed when infants were not crying (7.6 units compared to 7.9 while crying); an opposite trend was observed for pumps, with less movement units while crying compared to non-crying steps (5.8 and 6.8 units respectively). For individual response profiles, see Appendix C, Figure 54.

6.4 Discussion

6.4.1 Forward stepping was not affected by optic flow or arousal
This chapter aimed to assess the changes of optic flow added to a Dycem enhanced, moving treadmill belt on a kinematic level to determine whether optic flow had a subtler influence on stepping than could be observed in step rate alone. In terms of step and pump length, no evidence was found for changes in forward progression in response to optic flow or arousal. These factors may not influence the length of the movement, but it is also possible that the experiment did not find differences due to type II error. Note that due to data attrition, sample size was low in the step length analysis (n = 13) and the pump length analysis (n = 7). Together with the high level of variability and the fact that the expected effect may not be very large, the experiment may not have enough power to detect differences. However, individual plots showed no general trends in the data and additionally, the video-coded analysis of the same data (Chapter 5) did not find a shift from pump to step or vice versa as a function of optic flow and arousal. This analysis confirmed that even within the step and pump categories themselves, optic flow did not tend to change the length of the movement. Congruently, Barbu-Roth et al. found little evidence for kinematic change in air-stepping when neonates were exposed to optic flow - except for hip and step extension velocities (2013).

The lack of pervasive effects of optic flow on treadmill-elicited stepping may be because the treadmill is a very strong stimulus which leaves little room for improvement from further sensory stimulation (Chapter 5) and determines the length of the step though mechanical pull. So far, increased step lengths have been reported in new walkers and suggest increased integration of the foundation skills that support walking. In other
words, while toddlers reproduce multiple step cycles - at precarious balance and with the aim to control forward momentum with their stepping motions - their neuromuscular system fine-tunes complex muscle synergies that support increasingly narrow, longer step trajectories (Adolph et al., 2003). Newborns, on the other hand, do not tend to make large forward steps unless they are on the treadmill (Siekerman et al., 2015). The individual profiles in Appendix C showed that step length was constrained within a bandwidth between approximately 10mm and 70mm with a range of 60mm. Within this range, individual responses to optic flow or arousal showed infants’ natural variability, but this variability was not due to optic flow or arousal. This suggests that the length of the step was more likely determined by mechanical pull of the treadmill rather than by optic flow or arousal in this experiment, particularly because it featured a high-friction cover to the treadmill belt. This may have increased the ability to pull the leg even more.

The strength of the mechanical pull of the treadmill also helps to further understand the results from Siekerman et al. (2015): the increase in typical, adult-like steps with high arousal was probably not due to an increase in step length. In fact, in this study, typical steps increased because steps had better stance phases and/or further forward placed touch-downs: the typical step parameter amalgamated both characteristics in one category. Long step lengths were not inherent in ‘typical’ steps even if the touch-down was placed further forwards, especially if they only had a short stance phase. Similarly, a qualitatively good stance phase may have not been long if touchdown occurred behind the hip. Therefore, arousal may not have affected step length in that study. However, it possibly made steps more ‘typical’ because the increased overall energy levels and movement speed (Hadders-Algra et al., 1993) made swing and stance more defined. In summary, the data show that arousal and optic flow do not affect step length in the presence of a strong treadmill stimulus, particularly when its friction is enhanced.

6.4.2 Inter-joint coupling tightened with high arousal and random optic flow
As described in the previous paragraph, Siekerman et al. (2015) found that arousal was associated with a tendency towards more typical stepping, but also ‘tighter’ interlimb coordination. Serial steps (loosely alternating steps with the contralateral step initiating within one second of the ipsilateral step) decreased in favour of alternating steps (steps that alternated without lag) when newborns were more aroused. Evidence from animal treadmill stepping studies suggested that alternation is regulated within the spinal cord; spinalised cats could step with coordinated hindlimb alternation (Hiebert et al., 1996) -
without input from the brain. Although this mechanism has not been clearly demonstrated in humans, some researchers propose it is the primary cause of stepping in newborns (Yang et al., 1998a). Because of the complexity and variability observed in neonatal stepping, this explanation falls short. However, Siekerman et al. (2015) cautiously suggested that perhaps control level is brought to a more primitive state under the influence of high arousal, producing steps of a higher stereotypicity and automaticity - with higher levels of alternation and smoother, better steps.

Although peak flexion and peak extension rates have not been previously used to describe coordination, these measures give an impression of when joint movement is greatest. Timing the main phase of hip movement simultaneously with the main phase of knee movement illustrates a high degree of coupling between joints. In this study, inter-joint coordination tended to become more synchronous with higher arousal. Coupling between maximal angular velocity of peak knee and hip (peak extension rates) was tightest when infants were crying. Thelen (1986b) saw that de-coupling of joint motion increased as infants developed; her youngest participants (approximately one month of age) showed tightly synchronised peak knee and hip flexion, whereas older infants were able to de-couple movement in the hip and knee.

In this experiment, we saw less ‘mature’ intra-limb coordination with higher arousal. This was converse to previous findings (Siekerman et al., 2015), where arousal was associated with smoother and more adult-like steps, and increased de-coupling between legs. However, the relationship between neural input and end-effector behaviour is complex. This was demonstrated by the fact that the improvement of the kinematics of stepping in infants and walking in toddlers was not observable in the neuromuscular concomitants (Chang et al., 2006, Teulier et al., 2012). It is possible that similar effects at the neural level affected intralimb and interlimb coordination differently. Although one would intuitively expect that intra-limb coordination would have followed interlimb coordination in its de-synchronisation, consider that interlimb alternation itself became tighter in Sickerman et al.’s experiment (2015). Within steps that were already alternated, the anti-phase relationship became stronger. In the current experiment, the in-phase relationship strengthened, perhaps reflecting that phase relationships were attracted towards less variable coupling within their domain of preference.

However, it may be too simple to draw a parallel for inter and intralimb coordination, because the underlying factors determining optimal movement are very different for both: in interlimb coordination, the aim is to maintain a base of support and for inter-joint...
coordination to optimise motion of the hinged pendulum. When considering angular velocity graphs from healthy adults (Granata et al., 2000), peak knee flexion rate occurs at approximately 60% of the walking cycle (just after toe-off) whereas peak hip flexion rate occurs closer to 70% of the cycle. It appears that the hip and the knee do not reach peak flexion rate at exactly the same time in adults, although they both occur at the start of the swing phase. Peak extension rate seems to be more decoupled, and occurs at 20% of cycle time for the hip and at 90% of cycle time for the knee. Clearly, inter-joint coordinative relationships are more complex than the opposite phasing seen in intralimb coordination, suggesting that they need considerable fine-tuning before becoming optimal. Measures of variability (Clark and Phillips, 1993) may be more suitable to determine the level of maturity of the phase relationships. Nevertheless, the data suggest a freezing of degrees of freedom occurs in joint motion with high arousal. Interestingly, intralimb coordination was also affected by optic flow. The coupling between peak hip and knee flexion rate - i.e. the minimal angular velocity of hip and knee - was more synchronous when random optic flow was combined with the moving treadmill. Peak flexion rate was most de-coupled when the optic flow moved congruous to the treadmill, suggesting that the coherence between the treadmill and the optic flow allowed infants ‘unfreeze’ degrees of freedom within the movement of their legs. Overall, the observations of joint decoupling under congruent sensory conditions and low arousal suggested that certain sensory contexts allow for more room for adaptability and variability.

6.4.3 Effects of optic flow on the hip extensor
The ability of newborns to step is a measure of general available muscle energy as it reflects the ability to lift the leg against gravity (flexion) or sustain the body weight (extension). A recent paper discussed the role of the extensor muscles in stepping. Anderson and colleagues proposed that neonatal stepping is a manifestation of the extensor-supporting response and the terrestrial variety of intra-uterine ‘locomotion’ (Anderson et al., 2016). Particularly the extensor may have experienced considerable practice in the womb, explaining the ability to step in the newborn period. When infants gain more weight than their leg muscles can support in the upright position, the expression of upright stepping decreases considerably (Thelen and Fisher, 1982). When contemplating the importance of the extensors in upright locomotion, could the activity of the extensor action be sensitive to modification by visual stimuli as early as birth?
Jouen et al. (2000) found strong postural responses to faster optic flows in newborns, so it is plausible that steps may also be influenced by visual stimulation. Although no other effects were found in response to optic flow, an increase in maximal hip extension was observed when the treadmill was random compared to the congruent condition. Conversely, a previous air-stepping experiment showed increased hip and step extension rate in response to translating optic flow compared to a no optic flow-tactile condition (Barbu-Roth et al., 2015) in two-month-old babies. The fact that newborns extended less during congruent conditions seems therefore contradictory. However, the current experiment featured a moving treadmill and newborns, whereas Barbu-Roth’s experiment supported two-month-old infants in the air, which may have caused subtle differences. Perhaps random optic flow in combination with a translating treadmill confused the newborns, or subtly excited them – without inducing crying. This may have caused them to extend the leg more strongly. Congruent optic flow may have reduced conflict in the visuo-motor link, causing infants to step less vigorously. Even if the relationship between optic flow, treadmill stimulation and the extensor-supporting response is not entirely clear, it appears that infants are sensitive to the lack of congruency in visual and tactile-proprioceptive stimulation from a young age (also, see Anderson et al., 2016).

### 6.4.4 Arousal also affected step extension

The relationship between arousal and extension seems clearer. Significantly greater minimal and maximal hip angles were observed during steps made while crying - without a significant change in ROM - suggesting that extension was greater throughout the entire step. Additionally, flexion and extension rates were greater - during stance and during the whole cycle - at times in which infants were crying. When considering Hadders-Algra’s observations that increased arousal improves muscle contractions and movement vigour in general movements (1993), it is not likely that the extensor muscle became more active in isolation. However, it is possible that inter-muscular strategies became more efficient. This is supported by the fact that hip extension velocities were also significantly higher for steps made during crying. It is also possible that overall muscle activity afforded infants to accept more weight on the legs during stepping, causing better extension angles at the end of the step. We tentatively propose that a change in efficiency between the hip flexors and extensors and increased weight acceptance may be the cause for the extensor bias, although the examination of muscle behaviour in Chapter 6 must be considered to confirm this idea.
Arousal affected the knee somewhat differently; we observed an increased ROM during steps made while crying, although this was not associated with significantly more flexion or extension. This suggested that although the individual ROMs calculated during each step were consistently greater while infants were crying, absolute peak angles shifted from smaller to larger more randomly. Additionally, the knee flexed faster overall when infants cried. The results support Hadders-Algra’s findings of higher vigour and increased burst amplitudes (1993). The differences between hip and knee behaviour may be explained by the fact that development tends to happen in a roughly cephalocaudal direction (Woollacott and Shumway-Cook, 1990), and perhaps the hip was more sensitive to arousal level. Additionally, joint actions have different purposes in the step cycle. Fast knee flexion may be more easily achieved than fast knee extension during the entire cycle, possibly because knee extension is constrained by co-contraction to stiffen the leg during the stance, or because knee flexion is assisted by the elastic properties of the naturally hyper-flexed newborn hip.

6.4.5 General implications
Overall, it appears that joint action was sensitive to modification by optic flow, but more strongly by arousal. Although optic flow and arousal did not cause differences to step length or shifts from pumping to stepping, inter-joint coupling was affected by these external and organismic constraints. The coupling between peak hip and knee flexion rate – i.e. the minimal angular velocity of hip and knee - was more synchronous when random optic flow was combined with the moving treadmill. The fact that peak flexion rate was most de-coupled when the optic flow moved congruously to the treadmill suggested that despite the pervasive effect of the tactile stimulation by the treadmill over the optic flow, visual and tactile-proprioceptive stimuli reinforced each other to produce more adaptable coordination in this specific case.

It is suggested in the literature that tactile stepping can be elicited more easily when infants are in relative extension. Barbu-Roth linked infants’ stepping latency at approximately two months to their inability to extend the legs against the ground at this age (2015). The current study shows a tendency for increased hip extension during crying. Is there a potential link between arousal, hip extension and step rate? Perhaps it is not only due to better muscle energy that newborns step more when they are more aroused (Siekerman 2015), but it is possible that the relative improvement of hip extension influences stepping rate reciprocally. Interestingly, Barbu-Roth et al. (2015) also reported
greater step extension and hip extension velocities, in their case for tactile and air-away conditions. It is possible that outside of the influence of the treadmill, sensory stimulation can prompt the infant to extend more. The notion that relative extensor improvement as a mediator for increased stepping is interesting in the light of the proposition that infants are unable to step when their extensor strength becomes mismatched to their body weight. Treadmill training may maintain extensor participation during stepping as well as fortifying it, so that when infants become heavier, they can remain stepping and further explore coordinative patterns for stepping.

It is important to note that particularly for the optic flow analysis, the changes were not widespread and where they were significant, they were not substantial. There was a difference of 11.7% in the medians for flexion synchronicity, and 4.7 degrees for peak hip extension. However, for both variables, the response trended in the same direction for 75% of the infants. Although this may not affect the immediate execution of stepping, it does confirm the ability of optic flow to subtly affect step execution on the treadmill in most infants, particularly where responses otherwise are very variable. It may be that the effects will become more pronounced with practice, or that they reflect tenuous differences that fit into a larger cohort of changes that occur when newborns experience congruous or conflicting optic flow.

Interpreting these results poses an interesting question: does an improved extensor-support response reflect more advanced stepping behaviour or is it an expression of more automated control? Even if automated control was not a factor, high arousal and random optic flow did appear to constrain the kinematics of the steps. Considering that variability offers the best opportunity for selection of appropriate neuromuscular strategies, triggering steps through high arousal may not necessarily offer optimal opportunities for neuromuscular strategy selection. Offering an experience which allows exploration - even if only to a small degree - will enhance the quality of the training through the effects of perception and action coupling (Thelen, 1995). If the choice fell to increasing quantity, for example through arousal, stepping may become less flexible because infants attuned a narrower range of movements solutions during stepping while crying.

It is possible that adaptability in this early stage may not yet be of great importance because newborns and young infants don’t need to be highly adaptable to step. Additionally, flexibility may only be affected to a very small extent when considering newborns’ naturally high level of variability. Nevertheless, it is important to remember that early experience lays the foundations on which future functionality is built and
therefore, variability should be protected as much as possible. Finally, there is one last consideration in terms of the use of arousal to improve stepping: crying is a signal of discomfort or distress and should be minimalised in newborns, as sustained crying may have far-reaching effects (Douglas and Hill, 2013). High arousal may therefore not be an optimal context for interventions.

6.5 Limitations

Some limitations must be considered. Firstly, due to the delicate nature of the subject group, the correct placement of markers was difficult and subsequently, some markers fell off during testing, or were obscured, leading to data loss. This led to the de sample for final analysis becoming smaller than the original 20 participants (see Appendix B, Figure 19 for an overview of included data). Additionally, the step numbers differed across the conditions causing parameter means to be computed from differing entry sizes. For example, mean joint angles for a whole subject may consist of only one joint angle for one condition, but may be computed over more joint angles for the other conditions. Additionally, the analysis lacks measures of variability. High variability was pervasive throughout the experiment, decreasing the power of the statistical analysis. However, variability is inherent in neonatal behaviour, and a detailed report of the data would have further confirmed the lack of stereotype in newborn stepping. The profiles in Appendix C illustrate these individual differences.

6.6 Conclusion

In summary, there are some changes in kinematics in response of optic flow. There were differences between the responses in intralimb coordination and the level of extension during the step between random and congruent conditions, with congruent optic flow attenuating extension and desynchronising coordination. Arousal showed more categorical effects, with increased range of motion, joint angle peaks and increased synchronisation for higher arousal, with some differences between hip and knee. Although not strong, there was some evidence that stereotypy decreased as a result of lower arousal and congruent sensory conditions, which might be further supported by EMG analysis. This may have clinical implications; congruent optic flow and low arousal
may contribute to an environment that is optimal for perception-action cycles and allowing for unfreezing of degrees of freedom.
Chapter 7:

ELECTROMYOGRAPY OF STEPPING
AT DIFFERENT TREADMILL SPEEDS
AND ON THE TREADMILL WITH
ADDED OPTIC FLOW
7.1 Introduction

Infant motor behaviour has been extensively researched, particularly their tendency to make step-like movements in response to being supported upright on a surface (McGraw, 1940, Thelen and Ulrich, 1991, Thelen and Fisher, 1982, Thelen and Cooke, 1987, Yang et al., 1998a, Teulier et al., 2012). However, relatively little research focuses specifically on the newborn period, between birth and two weeks. Although infant and newborn stepping resembles adult walking, newborns do not support their own weight, keep their legs relatively flexed steps during the step cycle and show little forward progression (Forssberg, 1985). The behaviour is seen as a precursor to voluntary stepping later in infancy and with independent walking due to the continuity of its kinematics (Thelen and Cooke, 1987) and its response to training (Zelazo et al., 1972). Viewpoints on how stepping is controlled diverge. One approach attributes the response to a central pattern generator (CPG) which regulates neuromuscular locomotive patterns under the influence of feedback loops and reflex action between the limbs and the spinal cord (Dietz, 2003, Forssberg, 1985, Yang et al., 1998a, Dominici et al., 2011). According to this theory, the cortex takes over the control of stepping as the infant develops and begins to step voluntarily. Others (Thelen and Ulrich, 1991, Barbu-Roth et al., 2009, Teulier et al., 2009) view stepping from a dynamical systems perspective, where global aspects such as anthropometrics, environmental contexts and experience play a greater role in the expression of behaviour. During development, the refining of motor responses occurs as a function of trial and error, with multiple transitions occurring when combinations of functional behaviours become accessible through the improvement of strength, control, physical growth and sensory attuning. For example, stepping typically disappears in the third month of life. However, infants continue to kick when supine. According to this theory, stepping is expressed as kicking during a period in which infants’ strength-to-weight ratio becomes unfavourable (Thelen et al., 1982, Thelen and Fisher, 1982) and they can no longer support their own weight when upright (Anderson et al., 2016).

7.1.1 Clinical implications

The clinical implications of the two viewpoints are important; dynamical systems theory emphasises the role of experience in infant development, which has led to successes in early interventions for infants at risk of a delay in the attainment of walking (Looper and Ulrich, 2010, Ulrich et al., 2008, Wu et al., 2010, Wu et al., 2007, Ulrich et al., 2001). In these studies, considerable progress has been made by enhancing sensory stimuli. The
principal example is their use of the treadmill, which introduces movement to the legs. The treadmill makes stepping more frequent, even during times in which stepping cannot typically be evoked (Thelen, 1986b). Additionally, it appears to advance interlimb coordination in older infants (at around seven months - Thelen, 1986b). Siekerman et al. (2015) showed that the treadmill has a stimulating effect in even the youngest newborns. A second example is the use of visual stimulation. Optic flow has been successfully employed to enhance stepping on the treadmill in older infants (Moerchen and Saeed, 2012) and in infants with spina bifida (Pantall et al., 2012), although the effects were stronger in older infants from seven months of age. Optic flow alone was also able to elicit air stepping and stepping on a non-moving surface in newborns (Barbu-Roth et al., 2013, Barbu-Roth et al., 2009, Barbu-Roth et al., 2016). However, optic flow did not appear to have effects on step rate when newborns were also on a moving treadmill (Chapter 5). Pantall and colleagues addressed this question (Pantall et al., 2012), as they saw clear neuromuscular adaptations in response to optic flow in the youngest group of infants, but no increase in step rate. These authors proposed that ‘although younger infants were able to translate the visual input into a motor unit activity, they were unable to further develop the response into an organized patterned movement’ (p. 1684). When considering this interpretation, it is possible that changes in newborn stepping behaviour in response to optic flow on the treadmill were only observable at the neuromuscular level.

7.1.2 The study of neuromuscular patterns in stepping

Very few studies examine neuromuscular patterns of stepping newborns directly after birth. Dominici et al. (2011) studied neuromuscular patterns in a large sample of newborns of three days old in an overground stepping study, and found that two basic sinusoidal patterns accounted for nearly 90% of the variance in newborn muscle activation, which were similar to those found in animals. With the development of stepping into independent walking, more complex, pulsatile patterns overlaid these, although the basic patterns were retained. Other research of muscle activation during overground stepping found initial high levels of antagonist coactivity across each developmental stage of walking, which decreased with progress (Okamoto and Okamoto, 2001, Okamoto et al., 2003). Yang at al. (1998a), however, found that reciprocal activity was evident regardless of age, which was presented as evidence for innate patterns for stepping. Others (Forssberg, 1985, Dominici et al., 2011, Yang et al. 1998) proposed that
although muscle strategies were typically variable, some aspects of neuromuscular behaviour were patterned and predictable. It should be noted that in the majority of these studies, the measures of coactivity or predictability were poorly quantified or difficult to reproduce. Additionally, few studies focused on newborns alone. Another drawback was that no measures of variability were reported, and conclusions may have been drawn from aggregated step cycles. However, considerable inter and intra-individual variability and coactivity are typical for infant muscle activation, as was demonstrated by developmental studies in other motor skills that included reproducible measures for these parameters. For example, Spencer and Thelen (2000) found high levels of coactivity when infants learned to reach. They suggested coactivity was an adaptive control strategy, as it stiffens the joints and makes it more resistant to internal and external perturbations. The timing of muscle activity becomes more fine-tuned with experience. More recently, some well-quantified, reproducible EMG studies that included measures of variability and predictability were performed on the effect of walking experience in toddlers (Chang et al., 2006), on the effects of sensory augmentation in spina bifida (Pantall et al., 2012) and on age-related changes before onset of walking (Teulier et al., 2012). Interestingly, these studies showed pervasive variability and coactivity, with a tendency for kinematics to stabilise before neuromuscular patterns become more efficient and predictable.

7.1.3 Gaps in the literature
Currently, no studies have examined EMG in newborns on the treadmill. As Chapter 4 (Siekerman et al., 2015) found that newborns can step on the treadmill and are sensitive to speed and arousal, further detail is needed to support possible interventions at ages younger than the current standard – for example, from when infants able to sit (approximately 10 months of age - Ulrich et al., 2001), or from when cortical control has been established (Schlittler et al., 2011). Additionally, an explanation of why optic flow affected kinematics but not step rate in newborns who stepped on the treadmill may be found in the study of muscular activity (see Chapters 5 and 6). Finally, the effect of arousal on muscle activation during stepping requires more in-depth examination. Siekerman et al. (2015) found that highly aroused (i.e. crying) infants appeared to produce smoother, more adult-like steps with tighter interlimb coordination and proposed that arousal does not only improve movement vigour (Hadders-Algra et al., 1993) but also makes stepping more ‘stereotypical’ or automated. Kinematic analysis of stepping with optic flow on the treadmill showed more extension during high-arousal stepping. It was
not clear if this was due to increased weight acceptance by infants, or to a relative improvement of extensor activity.

7.1.4 Aims
The current report aims to describe the effects of treadmill speed, optic flow and arousal on muscle activation in stepping. Chang noted the importance of thorough quantification of variability and reproducibility (2006). Thus, the current method was chosen on the basis that can it reflect differences but also give a measure of predictability and variability. The same method was used by Pantall et al. (2012) and Teulier et al. (2012) in infant stepping, and the current study will therefore add knowledge of the newborn period to the overall story of neuromuscular behaviour in the development stepping throughout the first year of life into toddlerhood.

Considering that improvement of kinematics preceded stabilisation of muscle strategies (Chang et al., 2006), it is hypothesised that muscular activation patterns will be even less defined and predictable, and more coactive than those reported for one-month-old infants (Teulier et al., 2012). It is therefore possible that no patterns or changes can be observed at this age. However, Pantall et al. (2012) observed neuromuscular sensitivity to sensory stimuli at in two-month-old infants with spina bifida, and it is possible that infants with typical development may show similar responses at birth. Additionally, if spinal pattern generators were involved in stepping, the most likely time to find evidence for this would be directly after birth. And if automaticity and stereotypy was increased with high arousal in newborns, evidence of a shift in control may be observable as an increased organisation of neuromuscular strategies, for example through higher probability of activation, more reciprocal activation and more defined, adult-like probability profiles.

Furthermore, results from previous chapters suggested that changes at the gross movement level most likely occurred as a function of mechanical pull. Perhaps there are indications for this effect at the neuromuscular level, for example, through increased muscle activity or changes in activation timings with higher treadmill speed. Additionally, any changes occurring due to optic flow may not have been strong enough to reach the gross movement level, but may have been observable within the EMG. In this light, it will be interesting to examine if the kinematic changes found in Chapter 6 can be observed in the muscle signal, for example through increased extensor activity.
7.2 Methods

Two experiments were performed to generate the data in this study. The first experiment (Experiment 1) collected EMG data in two day old infants on a treadmill at different speeds (static through 0.234 m • sec\(^{-1}\)); the second experiment (Experiment 2) recorded the data in a similar subject group but with different optic flows projected on a slowly moving, Dycem enhanced treadmill (0.134 m • sec\(^{-1}\)).

7.2.1 Participants and Ethics

Twenty-eight (Experiment 1) and 24 (Experiment 2) infants were recruited from the maternity ward of the Port Royal Maternity Hospital in Paris. Healthy infants born with an uncomplicated delivery, a minimum term of 38 weeks, an Apgar score of 10 at the fifth minute and a minimum birth weight of 2500 grams were selected. Four (Experiment 1) and four (Experiment 2) infants were excluded from the final sample because these infants fell asleep during the experiments. An additional three infants were excluded from the first experiment; one for excessive crying and two because they performed fewer than 10 steps during the entire experiment. The final sample included 41 newborns (23 m, 18 f; birth weight 3310 (±440) grams; age 2.9 (±1.4) days; mean term 39.8 (±1) weeks). Ethical approval was obtained from the ethics medical board of Paris-Ile de France and parents and caregivers gave informed consent for infant participation.

7.2.2 Preparation

Infants were tested after feeding, when they were alert and not crying. After arrival in the movement analysis lab, infants were undressed to their undergarment and diaper. The electrode sites on the legs were cleaned with benzoic tincture and hypoallergenic Ag/AgCl dual electrodes (Noraxon Inc., Scottsdale, AZ) were placed bilaterally on the muscle bellies of the Rectus Femoris, Biceps Femoris, Tibialis Anterior and Lateral Gastrocnemius (see Figure 26). Locations of the muscle belly were found by palpation. The ground lead was attached to an area on the thigh without major muscle groups, as it was too large to attach it to a bony prominence. To decrease movement artefact, leads were fixated with tubular net bandage and supported along the arm and back of the tester. At the end of the testing, the electrodes were carefully removed with paraffin oil and a cotton bud.

Additionally, for Experiment 2, infants were dressed in a black sleeveless undergarment. Markers were placed, bilaterally, on the hips, lateral knees and lateral ankles. Further,
markers were placed on the neck at the location of the C7, and bilaterally on the shoulders, elbows and the centre of the dorsal wrists (see Chapter 6).

7.2.3 Presentation of the stimuli
In both experiments, infants were supported on an infant sized treadmill (belt surface 0.31m x 0.59m). In Experiment 1, infants experienced four different treadmill speeds (static treadmill, slow, medium and fast treadmill; see Chapter 4 for a detailed description). In Experiment 2, infants experienced four different optic conditions on a treadmill covered with Dycem and moving at the slow speed used in Experiment 1 (no optic flow, random optic flow, optic flow congruent the treadmill and optic flow faster than the treadmill). The different conditions were offered for one minute each, and in random order. For more detail on the presentation of the optic flow, see Chapter 5.

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1. Rectus Femoris (left and right)
2. Tibialis Anterior (left and right)
3. Biceps Femoris (left and right)
4. Lateral Gastrocnemius (left and right)

**Figure 26: Bilateral electrode distribution**
7.2.4 Testing procedure
Infants were then supported under the armpits by the experimenter’s hands (upright and angled slightly forwards, with the head and neck supported by the thumbs and the index finger), with their feet in contact with the treadmill belt. The four different conditions were presented in random order in four one-minute trials. Infants were repositioned at the front end of the treadmill if they had not responded to the treadmill for more than 10 seconds, to allow the hip to move through flexion and extension. If infants showed signs of high distress, e.g., desperate crying without pauses, or if the parents indicated their infant was too stressed to continue, they were removed from the treadmill before the end of the trial. Missing trials were not repeated because the newborns were only in an optimal condition for testing for a brief period of time. Note that due to the amount of care needed when testing newborns, complete blinding of the experimenter was impossible.

7.2.5 Data capture
Video cameras were placed around the infant; in Experiment 1, one camera was placed on the right and perpendicular to the infant (Sony DCR-HC26, Sony Corporation, Tokyo, Japan; collecting at 30 Hz), and one directly ahead of the infant (Sony DCR-HC26; collecting at 30 Hz). In Experiment 2, the camera configuration was the same, but one additional camera was placed to the left of the infant; additionally, both side view cameras collected video data at 60 Hz (Sony CHDR-CX160, Sony Corporation, Tokyo, Japan). The EMG signal was captured with Noraxon Myosystem 1400 (Noraxon Inc., Scottsdale, AZ) sampled at 1000 Hz. It was pre-amplified with a factor of 10 and pre-filtered with an inbuilt 10 Hz second order high pass filter and a 500 Hz eight order low pass filter.

7.2.6 Data reduction
Video data were analysed frame-by frame in SiliconCoach (SiliconCoach Ltd, Dunedin, New Zealand, version 7-0-2-2) to analyse the frequency of the movements and to record their start and finish. Periods with excessive tester movement (e.g., during repositioning of the infant) or touches by a parent (e.g., to encourage or calm the infant) were excluded from the analysis because they led to movements that were not generated by the infant.
7.2.6.1 Movement coding
Movement categories were based on an earlier experiment on neonatal treadmill stepping (Siekerman et al., 2015) with two main movements: pumps and steps. Pumps included vertical flexion and extension cycles, regardless of whether the foot was in contact with the belt at the start or end of the movement. Steps displayed locomotive potential, with the foot travelling forward for more than half a foot length during swing, whereas pumps consisted of vertical movement. Only start, touchdown and finish time of steps were utilised for this analysis.

7.2.6.2 Arousal coding
Arousal was coded using a combination of all the video footage (from the left, right and left-front), depending on the direction of the newborn’s face. The scheme used to code arousal was identical to that used in the previous study (Siekerman et al., 2015) and based on Thelen’s six-point scale adaptation (Thelen et al., 1984) of Prechtl’s behavioural states (Prechtl, 1974). Arousal was sampled at five second consecutive intervals, recording 1) gross body movement, 2) if eyes were open or closed and 3) level of vocalisation. Precise mapping between stepping and arousal made it possible to examine whether the stepping parameters changed between less aroused, non-crying states (state 4) and highly aroused, crying states (state 5 and 6). This was done by assigning a state code to each step and pump, based on the arousal level of the five second time window in which the majority of the movement occurred.

7.2.6.3 EMG Processing
EMG was further processed using a custom-built MATLAB program (MATLAB R2012b, MathWorks, Inc. Natick, Massachusetts, USA), which filtered each muscle in each trial with a sixth order Butterworth filter with cut-off frequencies of 10 and 300 Hz. This frequency was based on visual inspection. An additional Hampel filter (DX 400) was utilised to attenuate unexpected regular high spikes observed in Experiment 2. A 50 Hz notch filter was added to filter out noise from surrounding electrical devices. The data was rectified and then transformed to ‘on-off’ sequences by moving a 220 ms integration window across the signal frame by frame. At the end, a median filter of 160 ms was used to remove outlying values. If the average signal exceeded both alert and a validation threshold, the middle frame of the 220 ms window was considered to be ‘on’, with the
start of the ‘on’ sequence starting at the alert threshold. Only when the signal consequently decreased to below the alert threshold, it was considered to be ‘off’ again. In order to calculate those thresholds, a baseline value corresponding to the average of three to five seconds of muscle inactivity was calculated first. Then, a line between this baseline and the mean signal average over the entire trial was extrapolated. The threshold value (read on the y-axis) was modified depending on the strength or weakness of the activity of the trial by adjusting the x-value with a weighting factor based on the inverted signal-to-noise ratio (see Appendix E for more details). To obtain an alert threshold the same process was followed but the line was determined by an extra point: the median signal average over the entire trial. The size of the median value (high for high activity trials and low for low activity trials) adjusted the slope inclination to give more conservative thresholds when there was more activity during the trial.

The ‘on-off’ sequences were then used to calculate the ratio of activation (total frames ‘on’ divided by available time) and mean burst length. Further, the probability of activation (POA) was computed (Teulier et al., 2012, Pantall et al., 2012, Chang et al., 2006, Spencer and Thelen, 2000). This measure shows the likelihood that a muscle is active during consecutive time windows of a step cycle, with ‘1’ signifying that the muscle is always active and ‘0’ that the muscle was never active. For this, the step was normalised and divided into five windows for the swing phase and 10 for the stance phase. For each window, on/off status was determined. Then, the positives (muscle ‘on’)) were summed for all available steps from a given subject, for each window separately. The value for each window was then divided by the total number of available steps from that infant. For example, if a newborn made eight steps, and for the first window the muscle was ‘on’ during two steps, the POA value for that window would have been 0.25.

Additionally, ‘muscle state’ during swing and stance were calculated for each baby. For the four muscles captured, there are 16 possible combinations, ranging from ‘all on’ to ‘all off’, and any one, two or three muscle combination in between. The percentage of each muscle combination was calculated for all steps and integrated into averages for each infant. This method gives a detailed description of muscle contraction possibilities between all muscles. To obtain a real value of agonist-antagonist muscle contraction, a traditional method to calculate co-contraction was also utilised; coactivity between Rectus Femoris and Biceps Femoris, Rectus Femoris and Lateral Gastrocnemius and Tibialis Anterior and Lateral Gastrocnemius was computed using the formula (Winter 1990; Chang 2006):
\begin{equation}
Coactivity = 2x \frac{\text{Muscle A and B active}}{\text{muscle A active} + \text{muscle B active}}
\end{equation}

The formula will return values between 0 and 1, with 1 meaning complete co-contraction with muscle A and B always in simultaneous contraction.

7.2.7 Statistical analysis
Because Shapiro-Wilk tests showed that the data were not normally distributed in the majority of the parameters, nonparametric statistical tests were chosen (Friedman tests and Wilcoxon Signed-rank tests). When testing between the comparable conditions in the two experiments, independent t-tests were used, because the data were normally distributed here. For more details on the statistical methods, see Chapter 3.

7.3 Results
The results from the EMG analysis are described in three sections. The first section contains a purely descriptive illustration of EMG in newborn stepping on the treadmill with and without optic flow. Example EMG profiles are given to illustrate typical responses from two stepping newborns. A comparison of stepping on a static surface and a moving treadmill follows. The second section will report the effects of treadmill speed (Experiment 1) and optic flow on the moving treadmill (Experiment 2). The third section will examine the effects of arousal for both experiments.

7.3.1 SECTION 1: Neuromuscular patterns for stepping in the newborn
To illustrate the nature of neuromuscular behaviour during stepping, example EMG signal of an infant who would be classified as an excellent stepper is depicted in Figure 27. Only this infant took more than two consecutive steps in the static condition (Figure 27a), and 12 sequences of two or more steps in the dynamic conditions (Figure 27b). To balance the data from this good stepper, Figure 28 shows EMG traces from a more average stepper. Because no stepping sequence of more than two steps could be obtained during the static condition for average steppers, two single steps from the static condition (Figure 28a) and one sequence of two steps on the same leg in the dynamic condition are depicted (Figure 28b). Both stepping sequences were taken while infants were in the same behavioural state (crying). From these example data, it is observable that contractions are not predictable across the steps of one subject or between those of different subjects.
Figure 27: EMG (filtered and rectified) from a good stepper across 2 conditions. A: EMG signal from stepping on the static treadmill (note that two is the maximal number of steps in sequence in this condition). B: EMG signal from stepping on a moving treadmill (0.134 m • sec⁻¹). Signal was normalised; on the y-axis, signal is expressed as a percentage of the maximum (peak value produced by this infant in this condition). ST: stance phase; SW, swing phase.
Figure 28: EMG (filtered and rectified) from an average stepper across two conditions. A: EMG signal from stepping on the static treadmill (note that one is the maximal number of steps in sequence in this condition). B: EMG signal from stepping on a moving treadmill (0.134 m • sec-1). Signal was normalised; on the y-axis, signal is expressed as a percentage of the maximum (peak value produced by this infant in this condition). ST: stance phase; SW, swing phase.
The good stepper showed some evidence of phasic peaks in some steps, whereas the average stepper did not. In both subjects, there was evidence of all four muscles contracting together during certain periods, particularly during the transition from swing to stance.

The data from these two subjects was compressed to generalise these observations. As it is known that infant EMG is highly variable (Teulier et al., 2012; Pantall et al., 2012), a measure of variability or predictability is essential. Compressing the sample data into ensemble averages is not judicious, particularly without measures of data spread. A different approach, probability of activation (POA), determines the likelihood that a muscle is active during five time windows in the swing (0-20%, 20-40% etc.) and 10 during the stance (0-10%, 10-20%, etc.). This difference reflects the fact that the swing phase tends to be considerably shorter than the stance phase in newborns. Note that this approach does not describe amplitudes.

Figure 29 depicts POA profiles all steps made by the two newborns that supplied the data for the sample EMG profiles in Figure 27 and 28. The inter-individual difference is evident from these graphs: the muscles show very different responses between the two individuals. For example, in the good stepper, the peak around touchdown is observable in the increased likelihood of activation at the end of the stance phase (Figure 29a), and the average stepper shows greater likelihood of activation for the Lateral Gastrocnemius and the Rectus Femoris during mid-stance. Most noticeable is the range of the POA profiles: for the good stepper, POA ranges from approximately 0.3 to 0.65 across 69 steps, whereas POA ranges from 0.5 to 0.8 across 15 steps in the average stepper. This suggests that EMG was somewhat less predictable, or more variable, for the good stepper than for the average stepper.
High levels of inter-individual variability were confirmed across the entire first Experiment. Figure 30a shows the likelihood of activation from all cycles of all infants in Experiment 1. The POA profiles for the Biceps and Rectus Femoris and Lateral Gastrocnemius were quite flat, ranging from approximately 0.35 to 0.55. The likelihood that these muscles were active tended to be just below 50%; they were as likely to be active as inactive. Together with Figure 29, this graph indicates that the contributions of individuals cancelled each other out, suggesting high levels of interindividual variability. However, each of these muscles show a slight decrease mid-swing, illustrating a tendency for these muscles to be inactive during this time in some of the step cycles. The profile of
the Tibialis Anterior followed a different trend, with somewhat higher POA during mid-swing (peaking at approximately 0.65), and lower POA during the stance phase.

![Figure 30: Impression of POA for four muscles across two experiments, with a) Experiment 1 and b) Experiment 2](image)

In contrast, POA profiles in Experiment 2 were somewhat more defined, ranging from approximately 0.15 to approximately 0.7 for the Tibialis Anterior. Particularly for the mid-stance, the Tibialis Anterior was not active in approximately 85% of the steps. Additionally, profiles were similar across muscles, with higher activity during the swing and lower activity during the stance, suggesting that muscle activity was not only slightly
more predictable than for Experiment 1, but also that patterns for each muscle resembled each other.

7.3.1.1 Probability of activation for infants stepping on the static and the moving treadmill

To describe the effect of the moving treadmill on muscle activity patterns, EMG from all steps from the slow, medium and fast treadmill was treated as one group, and compared to EMG from steps on the static treadmill. Note that the groups were unbalanced: the dynamic group contained steps from three conditions (365 steps) and the static group contained steps made on the static treadmill alone (38 steps, see Table 20 in Appendix B for an overview of contributing data). The POA profile for the dynamic group is therefore likely more accurate.

Figure 31: A visual comparison between the POA profiles of the Biceps Femoris, Lateral Gastrocnemius, Rectus Femoris and Tibialis Anterior across the static and dynamic (slow, medium and fast treadmill) conditions.

POA of the Biceps Femoris, Lateral Gastrocnemius and Rectus Femoris appeared reasonably similar between steps made during static and dynamic trials (Figure 31), with slightly higher POA at the start of swing, a decrease during mid-swing, and higher POA during the transition from swing to stance. Only the Rectus Femoris decreased slightly at the end of stance in the static trial compared to the dynamic trials. However, there did not
seem to be a strong change associated with stepping on a moving treadmill compared to static stepping in these three muscles.

In contrast, the definition of the Tibialis Anterior, which was better than would be expected in infants, was even more pronounced during static steps. The muscle tended to be more active during the swing phase, peaking at approximately 85% for steps in the static condition compared to 60% in the dynamic condition. POA during static steps appeared higher during the swing and the beginning of the stance phase, suggesting an improvement in predictability or stability for static steps at this time.

7.3.1.2 Probability of activation between the first and second experiment

To describe the effect of optic flow on a Dycem-enhanced treadmill, POA profiles of the comparable conditions in Experiment 1 (slow treadmill) and Experiment 2 (No OF on the slow treadmill) are plotted in Figure 32.

![Figure 32: A visual comparison between the POA profiles of the Biceps Femoris, Lateral Gastrocnemius, Rectus Femoris and Tibialis Anterior across the two comparable conditions from the two experiments (slow treadmill speed in Experiment 1 and No OF condition in Experiment 2).](image)

For three of the muscles, the Biceps Femoris, the Rectus Femoris and the Tibialis Anterior, the profiles from the first and second experiment were reasonably similar, with some small differences at the beginning of the swing phase and/or at the end of the stance phase. The Lateral Gastrocnemius was somewhat different, with increased POA for
Experiment 1 during the swing and increased POA for Experiment 2 during the stance. POA for this muscle did not exceed 0.6 for either experiment at any time.

7.3.2 SECTION 2: The effects of sensory stimulation on EMG in stepping
The following section will describe, in detail, the effects of treadmill speed, and will follow up with the effects of optic flow on a moving treadmill. Variables under investigation are the probability of activation (POA), general measures across the entire trial regardless of stepping (activity ratio, muscle burst length and general coactivity), simultaneous activation of muscles across the step (muscle state) and antagonist coactivation during steps (step coactivity).

7.3.2.1 The effect of treadmill speed on EMG
Nonparametric tests (Friedman Tests with Wilcoxon Signed-rank tests for post hoc analyses) were used to look for significant differences between treadmill speed conditions (static, slow, medium and fast treadmill speeds). Activity ratio did not change under the influence of treadmill speed, and ranged from median 0.220 to 0.245 for the Biceps Femoris; from 0.240 to 0.250 for the Lateral Gastrocnemius, from 0.233 to 0.246 for the Rectus Femoris and from 0.256 to 0.271 for the Tibialis Anterior. General coactivity (GC) measured throughout the entire trial, and calculated for the classic antagonists, also did not change as an effect of treadmill speed. Medians ranged from 0.513 to 0.550 for the Rectus Femoris-Biceps Femoris pair; from 0.554 to 0.623 for the Rectus Femoris-Lateral Gastrocnemius pair and from 0.503 to 0.621 for the Tibialis Anterior-Lateral Gastrocnemius pair.

However, there were some significant effects for speed on mean muscle burst length (MBL, see Table 7). The initial Friedman analysis showed that the Biceps Femoris was significantly different across the treadmill velocities ($\chi^2 = 8.850$, $p = 0.031$) with the longest muscle burst happening on the static treadmill and the shortest bursts when the treadmill ran at its fastest speed. However, the difference was not significant after Bonferroni-Holm correction for multiple comparisons. For the Tibialis Anterior, burst lengths were significantly longer when the treadmill was static (1076.5 frames, or 1.08s) compared to when the treadmill was slow (780.0 frames, or 0.78s) or at medium speed (0.92s; $\chi^2 = 14.077$, $p = 0.003$). There were no further differences for muscle burst length.
Table 7: The effects of treadmill speed on general muscle activity (Biceps Femoris: BF; Lateral Gastrocnemius: LG; Rectus Femoris: RF and Tibialis Anterior: TA)

<table>
<thead>
<tr>
<th></th>
<th>BF</th>
<th>LG</th>
<th>RF</th>
<th>TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>χ²</td>
<td>1.575</td>
<td>0.600</td>
<td>3.171</td>
<td>3.000</td>
</tr>
<tr>
<td>N</td>
<td>16</td>
<td>16</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>p-value</td>
<td>0.665</td>
<td>0.896</td>
<td>0.366</td>
<td>0.392</td>
</tr>
<tr>
<td>Static Medians</td>
<td>0.241</td>
<td>0.250</td>
<td>0.233</td>
<td>0.262</td>
</tr>
<tr>
<td>Slow Medians</td>
<td>0.245</td>
<td>0.250</td>
<td>0.240</td>
<td>0.256</td>
</tr>
<tr>
<td>Medium Medians</td>
<td>0.241</td>
<td>0.240</td>
<td>0.246</td>
<td>0.269</td>
</tr>
<tr>
<td>Fast Medians</td>
<td>0.220</td>
<td>0.242</td>
<td>0.235</td>
<td>0.271</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Muscle burst length (frames)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
</tr>
<tr>
<td>LG</td>
</tr>
<tr>
<td>RF</td>
</tr>
<tr>
<td>TA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>General coactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF-BF</td>
</tr>
<tr>
<td>RF-LG</td>
</tr>
<tr>
<td>TA-LG</td>
</tr>
</tbody>
</table>

The effects of treadmill speed were observable in the likelihood of muscle activity. POA was significantly different between treadmill speeds for the Biceps Femoris and the Tibialis Anterior (see Figure 33). For the Biceps Femoris, this difference occurred during the stance cycle (at 20-30%: χ² = 8.065, p = 0.045 and at 80-90%; χ² = 8.719, p = 0.033), with the lowest POA during steps made on the slow treadmill and the highest POA during the fast speed (at the 20-30% stance window) and the static condition (for the 80-90% stance window). For the Tibialis Anterior, there was a significant difference during the swing phase (0-20%; χ² = 9.269, p = 0.026), with the lowest POA when the treadmill was medium and the highest POA when the treadmill was fast. Note that none of the differences were significant after Bonferroni-Holm correction: the effects were not strong. However, a trend was observable. P-values decreased around the transition from swing to stance in the Biceps Femoris, and again towards the end of stance: at the slow treadmill speed, the muscle was least likely to be active (see Tables 22 to 25 in Appendix D).
Figure 33: The effect of treadmill speed on muscle POA during steps

No significant differences between treadmill speeds were found for coactivity during stepping (SC) when measured with the traditional method, which calculates a measure of simultaneous activation between designated antagonists as a ratio of its overall activation (see Table 8). For the Rectus Femoris-Biceps Femoris pair, coactivity medians ranged from 0.591 to 0.662; for the Rectus Femoris-Lateral Gastrocnemius pair, coactivity ranged from 0.509 to 0.671 and for the Tibialis Anterior-Lateral Gastrocnemius pair, coactivity ranged from 0.385 to 0.635.

Table 8: The effects of treadmill speed on step coactivity. Biceps Femoris (BF); Lateral Gastrocnemius (LG); Rectus Femoris (RF) and Tibialis Anterior (TA).

<table>
<thead>
<tr>
<th></th>
<th>$\chi^2$</th>
<th>N</th>
<th>P-value</th>
<th>Static</th>
<th>Slow</th>
<th>Medium</th>
<th>Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Step Coactivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF-BF</td>
<td>0.120</td>
<td>5</td>
<td>0.989</td>
<td>0.623</td>
<td>0.617</td>
<td>0.662</td>
<td>0.591</td>
</tr>
<tr>
<td>RF-LG</td>
<td>1.400</td>
<td>6</td>
<td>0.706</td>
<td>0.509</td>
<td>0.590</td>
<td>0.671</td>
<td>0.598</td>
</tr>
<tr>
<td>TA-LG</td>
<td>2.143</td>
<td>7</td>
<td>0.543</td>
<td>0.385</td>
<td>0.396</td>
<td>0.635</td>
<td>0.585</td>
</tr>
</tbody>
</table>

However, when looking at the muscle state method, which calculates the absolute time of coinciding activity between all possible muscle combinations across a whole cycle,
some small differences were found (see Figures 34 and 35). Although no measure of coactivity, a difference was observed in the solitary activation of the Rectus Femoris during the swing phase ($\chi^2 = 10.172, p = 0.017$). The muscle was most active during the fast treadmill speed and least active during the medium treadmill speed, but the difference was small (median 1.8% compared to median 0%), and not significant after correction for multiple comparisons. In the stance, a significant difference occurred in the coactivity between Rectus Femoris and Lateral Gastrocnemius ($\chi^2 = 13.438, p = 0.004$), with the medium treadmill speed evoking the most simultaneous activation between these muscles, and the static and fast speed the least. The differences were not significant after Bonferroni-Holm correction.

Figure 34: Muscle state (combinations of simultaneously active muscles) across four treadmill speeds during the swing phase of the step. Significant differences are marked with *
7.3.2.2 The effect of optic flow condition on EMG

The effects of optic flow on the moving treadmill (no OF, random, congruent and faster; Experiment 2) were examined with nonparametric tests. Friedman tests were used to look for differences between the four conditions and Wilcoxon Signed-rank tests were utilised in the post hoc analysis. There were no significant differences between the different optic flow conditions for AR, MBL and GS (see Table 9). Ranges for AR medians were slightly higher than those in the treadmill speed analysis (Experiment 1), and ranged from 0.253 to 0.271 for the Biceps Femoris; from 0.259 to 0.270 for the Lateral Gastrocnemius; from 0.277 to 0.287 for the Rectus Femoris and from 0.283 to 0.298 for the Tibialis Anterior. This meant that the muscle was active, on average, for nearly a quarter of the trials. Mean burst lengths were similar to those of the treadmill speed analysis (Experiment 1) and ranged from 702.8 to 897.5 frames (0.7 to 0.9s) for the Biceps Femoris; from 733.5 to 879.0 frames (0.73 to 0.88s) for the Lateral Gastrocnemius; from 917.0 to 1012.2 frames (0.92 to 1.01s) for the Rectus Femoris and from 823.9 to 964.4 frames (0.82 to 0.96s) for the Tibialis Anterior.

General coactivity ranges were like those in Experiment 1 (treadmill speed analysis). For the Rectus Femoris-Biceps Femoris pair, coactivity ranged from 0.493 to 0.548; for the Rectus Femoris-Lateral Gastrocnemius pair, coactivity ranged from 0.525 to 0.583 and
for the Tibialis Anterior-Lateral Gastrocnemius pair, coactivity ranged from 0.511 to 0.530.

Table 9: The effects of optic flow on general muscle activity (Biceps Femoris: BF; Lateral Gastrocnemius: LG; Rectus Femoris: RF and Tibialis Anterior: TA)

<table>
<thead>
<tr>
<th></th>
<th>χ²</th>
<th>N</th>
<th>P-value</th>
<th>Static</th>
<th>Slow</th>
<th>Medium</th>
<th>Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Activity ratio</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>BF</td>
<td>5.141</td>
<td>20</td>
<td>0.162</td>
<td>0.254</td>
<td>0.253</td>
<td>0.271</td>
<td>0.258</td>
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<tr>
<td>LG</td>
<td>3.159</td>
<td>19</td>
<td>0.368</td>
<td>0.259</td>
<td>0.259</td>
<td>0.270</td>
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<tr>
<td>RF</td>
<td>0.792</td>
<td>20</td>
<td>0.851</td>
<td>0.285</td>
<td>0.287</td>
<td>0.280</td>
<td>0.277</td>
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<tr>
<td>TA</td>
<td>4.620</td>
<td>20</td>
<td>0.202</td>
<td>0.297</td>
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<td>0.298</td>
<td>0.293</td>
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<tr>
<td><strong>Mean burst length (frames)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BF</td>
<td>6.180</td>
<td>20</td>
<td>0.103</td>
<td>702.8</td>
<td>841.1</td>
<td>844.9</td>
<td>897.5</td>
</tr>
<tr>
<td>LG</td>
<td>1.295</td>
<td>19</td>
<td>0.730</td>
<td>784.2</td>
<td>733.5</td>
<td>826.6</td>
<td>879.0</td>
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<td>RF</td>
<td>1.140</td>
<td>20</td>
<td>0.767</td>
<td>994.7</td>
<td>1012.2</td>
<td>976.1</td>
<td>917.0</td>
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<tr>
<td>TA</td>
<td>3.480</td>
<td>20</td>
<td>0.323</td>
<td>862.2</td>
<td>911.7</td>
<td>964.4</td>
<td>823.9</td>
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<tr>
<td><strong>General coactivity</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>BF-LG</td>
<td>1.500</td>
<td>20</td>
<td>0.682</td>
<td>0.493</td>
<td>0.548</td>
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<tr>
<td>RF-LG</td>
<td>1.547</td>
<td>19</td>
<td>0.671</td>
<td>0.525</td>
<td>0.560</td>
<td>0.583</td>
<td>0.544</td>
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<tr>
<td>TA-LG</td>
<td>0.347</td>
<td>19</td>
<td>0.951</td>
<td>0.511</td>
<td>0.530</td>
<td>0.526</td>
<td>0.530</td>
</tr>
</tbody>
</table>

Optic flow affected the likelihood of muscle activation significantly. There were significant POA differences for all muscles except the Lateral Gastrocnemius (Figure 36). Differences for the Biceps Femoris occurred at the start of stance (0-10% of stance; χ² = 8.089, p= 0.044 and 10-20% of stance, χ² = 8.125, p = 0.043), with the highest POA in the random condition and the lowest POA in the faster condition. For the Rectus Femoris, the difference occurred during a time window just before the middle of the swing phase (20-40% of swing; χ² = 8.845, p = 0.031), with the highest POA in the congruent condition and the lowest POA in the random condition. Note that none of the above differences were significant after correction for multiple comparisons. However, they did fit into the general trend for muscles during steps on a moving treadmill with optic flow: faster optic...
flow tended to decrease POA at the start of stance for all muscles. For more details on the statistics, see Tables 26 to 29 in Appendix D.

For the Tibialis Anterior, significant differences also occurred during the start of the stance phase (0-10% of stance; \( \chi^2 = 9.844, p = 0.020 \)). For this time point, the Tibialis Anterior was significantly more likely to be active during the random and the congruent conditions compared to the faster condition. Towards the middle of the stance, the Tibialis Anterior was significantly more likely to be active in the no OF condition compared to the faster condition (20-30% of stance; \( \chi^2 = 10.947, p = 0.012 \) and 30-40% of stance \( \chi^2 = 8.300, p = 0.040 \)), although the latter difference was not significant after \textit{post hoc} correction for multiple comparisons.

There were no significant differences for step coactivity (see Table 10) between the different optic flow conditions, with medians ranging between 0.551 and 0.621 for the Rectus Femoris-Biceps Femoris pair; 0.500 and 0.615 for the Rectus Femoris-Lateral Gastrocnemius pair and 0.551 and 0.611 for the Tibialis Anterior-Lateral Gastrocnemius pair. There were also no significant differences for the percentages of coinciding activity between all possible muscle combinations (muscle state), suggesting that optic flow did not affect muscle coactivation levels in this experiment.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure36.png}
\caption{The effect of optic flow condition on muscle POA during steps}
\end{figure}
Table 10: The effects of optic flow on step coactivity. Biceps Femoris: BF; Lateral Gastrocnemius (LG); Rectus Femoris (RF) and Tibialis Anterior (TA).

<table>
<thead>
<tr>
<th></th>
<th>χ²</th>
<th>N</th>
<th>P-value</th>
<th>No OF</th>
<th>Random</th>
<th>Congruent</th>
<th>Faster</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Step Coactivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF-BF</td>
<td>4.597</td>
<td>14</td>
<td>0.204</td>
<td>0.551</td>
<td>0.621</td>
<td>0.609</td>
<td>0.596</td>
</tr>
<tr>
<td>RF-LG</td>
<td>5.300</td>
<td>12</td>
<td>0.151</td>
<td>0.615</td>
<td>0.564</td>
<td>0.568</td>
<td>0.500</td>
</tr>
<tr>
<td>TA-LG</td>
<td>0.771</td>
<td>14</td>
<td>0.856</td>
<td>0.590</td>
<td>0.611</td>
<td>0.551</td>
<td>0.603</td>
</tr>
</tbody>
</table>

7.3.3 SECTION 3: The effects of arousal on EMG

Arousal had stronger and more pervasive on muscle activity. AR, MBL and GC were affected by arousal in Experiment 1 and Experiment 2 (see Table 11). In the first experiment, all muscles were significantly more active when infants were crying. AR ranged from around 0.197 in the Rectus Femoris to 0.233 in the Tibialis Anterior when infants were not crying, whereas crying increased the ratio of activation to over 0.30 in all cases (ranging from 0.309 Lateral Gastrocnemius to 0.357 in the Tibialis Anterior), with all p-values at 0.001 or lower. For the second experiment, only the Biceps Femoris was more active when infants cried. The Lateral Gastrocnemius and the Rectus Femoris and Tibialis Anterior were not significantly different when crying or not crying; ARs ranged from 0.255 to 0.282 during non-crying times and from 0.263 to 0.328 during crying times.

MBL was less affected by crying. Only in Experiment 2, a tendency towards shorter burst lengths was observed in the Tibialis Anterior, the Rectus Femoris and the Lateral Gastrocnemius. The difference only became significant in the Lateral Gastrocnemius; muscle bursts decreased from 820.1 frames to 686.4 frames (from approximately 0.82 seconds to 0.68 seconds (Z = -2.229, p = 0.026).

GC was affected by arousal level in Experiment 1 only. The Rectus Femoris-Biceps Femoris pair was significantly more coactive when infants were crying (Z = -2.637, p = 0.008), increasing from 0.475 to 0.579. Rectus Femoris-Lateral Gastrocnemius pair also showed increased coactivity when newborns cried. Coactivity rose from 0.520 to 0.629 as a function of crying (Z = -2.068, p = 0.039).
Table 11: General muscle activity (activity ratio (AR), muscle burst length (MBL) and general coactivation (GC) by arousal level and for Experiment 1 (treadmill speed) and Experiment 2 (optic flow condition).

<table>
<thead>
<tr>
<th></th>
<th>Exp 1</th>
<th></th>
<th></th>
<th>Crying</th>
<th>Non-crying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z-value</td>
<td>N</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Exp 1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BF</td>
<td>-3.361</td>
<td>16</td>
<td>0.001</td>
<td>0.352</td>
<td>0.196</td>
</tr>
<tr>
<td>LG</td>
<td>-3.258</td>
<td>16</td>
<td>0.001</td>
<td>0.309</td>
<td>0.228</td>
</tr>
<tr>
<td>RF</td>
<td>-3.361</td>
<td>16</td>
<td>0.001</td>
<td>0.349</td>
<td>0.197</td>
</tr>
<tr>
<td>TA</td>
<td>-3.258</td>
<td>16</td>
<td>0.001</td>
<td>0.357</td>
<td>0.233</td>
</tr>
<tr>
<td>Exp 2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BF</td>
<td>-2.040</td>
<td>14</td>
<td>0.041</td>
<td>0.296</td>
<td>0.229</td>
</tr>
<tr>
<td>LG</td>
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<td>14</td>
<td>0.221</td>
<td>0.263</td>
<td>0.255</td>
</tr>
<tr>
<td>RF</td>
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<td>0.397</td>
<td>0.297</td>
<td>0.282</td>
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<tr>
<td>TA</td>
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<td>0.109</td>
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</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>BF</td>
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<td>0.163</td>
<td>762.4</td>
<td>765.7</td>
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<tr>
<td>LG</td>
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<td>16</td>
<td>0.352</td>
<td>809.0</td>
<td>1121.6</td>
</tr>
<tr>
<td>RF</td>
<td>-0.879</td>
<td>16</td>
<td>0.379</td>
<td>886.6</td>
<td>906.1</td>
</tr>
<tr>
<td>TA</td>
<td>-0.465</td>
<td>16</td>
<td>0.642</td>
<td>1052.5</td>
<td>1034.3</td>
</tr>
<tr>
<td>Exp 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BF</td>
<td>-0.282</td>
<td>14</td>
<td>0.778</td>
<td>727.8</td>
<td>719.6</td>
</tr>
<tr>
<td>LG</td>
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<td>14</td>
<td>0.026</td>
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<td>820.1</td>
</tr>
<tr>
<td>RF</td>
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<td>0.084</td>
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<tr>
<td>TA</td>
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<td>0.074</td>
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<td>Exp 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF-BF</td>
<td>-2.637</td>
<td>16</td>
<td>0.008</td>
<td>0.579</td>
<td>0.475</td>
</tr>
<tr>
<td>RF-LG</td>
<td>-2.068</td>
<td>16</td>
<td>0.039</td>
<td>0.629</td>
<td>0.520</td>
</tr>
<tr>
<td>TA-LG</td>
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<td>16</td>
<td>0.469</td>
<td>0.587</td>
<td>0.489</td>
</tr>
<tr>
<td>Exp 2</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>RF-BF</td>
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<td>14</td>
<td>0.124</td>
<td>0.520</td>
<td>0.487</td>
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<tr>
<td>RF-LG</td>
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<td>0.245</td>
<td>0.539</td>
<td>0.486</td>
</tr>
<tr>
<td>TA-LG</td>
<td>-0.722</td>
<td>14</td>
<td>0.470</td>
<td>0.490</td>
<td>0.496</td>
</tr>
</tbody>
</table>

Arousal affected the POA of the Biceps and Rectus Femoris in both experiments (Figure 37). Biceps Femoris was more active during steps when infants were crying, which became significant towards the end of the swing phase in Experiment 1 (during 60-80% of the stance phase; Z = -2.344, p = 0.019) and during mid-swing (40-60% of the swing phase; Z = -2.411, p = 0.016) for Experiment 2. Biceps Femoris was also significantly more active during crying steps towards the middle and end of stance for Experiment 2. Differences occurred at 50-60% of the stance phase (Z = -2.268, p = 0.023), at 60-70% of the stance phase (Z = -2.313, p = 0.021) and at 90-100% of the stance phase (Z = -2.271, p = 0.023).
POA was also significantly higher during crying steps for the Rectus Femoris, particularly in Experiment 1. For the majority of the swing phase (0% through 60% of the swing phase) and the majority of the stance phase (10% though 90% of the stance phase) the differences were significant. Because the large number of significant differences all show...
the same direction, they are not listed in detail here. For a comprehensive table of results, see Table 24 in Appendix D. In Experiment 2, the Rectus Femoris was only affected by crying during the stance phase; during 10% though 80% of the stance phase of steps made while crying, POA was significantly higher (for detail of the statistical outcomes, consult Table 28 in Appendix D).

Table 12: The effects of arousal on step coactivity in Experiment 1 and Experiment 2

<table>
<thead>
<tr>
<th>Step Coactivity</th>
<th>Exp 1</th>
<th></th>
<th></th>
<th>Crying</th>
<th>Non-crying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z-value</td>
<td>N</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF-BF</td>
<td>-1.098</td>
<td>12</td>
<td>0.272</td>
<td>0.657</td>
<td>0.604</td>
</tr>
<tr>
<td>RF-LG</td>
<td>-0.549</td>
<td>12</td>
<td>0.583</td>
<td>0.774</td>
<td>0.595</td>
</tr>
<tr>
<td>TA-LG</td>
<td>-0.734</td>
<td>13</td>
<td>0.463</td>
<td>0.460</td>
<td>0.595</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step Coactivity</th>
<th>Exp 2</th>
<th></th>
<th></th>
<th>Crying</th>
<th>Non-crying</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z-value</td>
<td>N</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF-BF</td>
<td>-1.352</td>
<td>7</td>
<td>0.176</td>
<td>0.516</td>
<td>0.429</td>
</tr>
<tr>
<td>RF-LG</td>
<td>-0.140</td>
<td>8</td>
<td>0.889</td>
<td>0.561</td>
<td>0.592</td>
</tr>
<tr>
<td>TA-LG</td>
<td>-1.521</td>
<td>7</td>
<td>0.128</td>
<td>0.511</td>
<td>0.667</td>
</tr>
</tbody>
</table>

No significant differences were found for traditional measures of coactivity as a function of arousal during stepping (see Table 12). Coactivity values were slightly higher in Experiment 1 compared to Experiment 2, with a maximum coactivity of 0.774 in the Rectus Femoris-Lateral Gastrocnemius pair in the first experiment, and a coactivity maximum of 0.667 for the Tibialis Anterior-Lateral Gastrocnemius pair in Experiment 2. Minima were 0.460 and 0.429 for the first and second experiment respectively.

However, the muscle state analysis found some significant differences. For Experiment 1 (Figure 39), the Tibialis Anterior and the Rectus Femoris showed simultaneous activity more often when newborns cried compared to non-crying steps during the swing phase ($Z = -2.746$, $p = 0.006$), whereas in the stance phase, there was a slight but significant increase in simultaneous activity between the Tibialis Anterior, Biceps Femoris and Lateral Gastrocnemius when infants were not crying ($Z = -2.090$, $p = 0.037$).

For the swing phase in Experiment 2 (Figure 40), Tibialis Anterior and Biceps Femoris were active together more when newborns cried ($Z = -1.988$, $p = 0.047$). In the stance phase of Experiment 2, Rectus Femoris and Biceps Femoris were slightly more active together when newborns cried ($Z = -2.353$, $p = 0.019$). Rectus Femoris, Biceps Femoris and Lateral Gastrocnemius were also more active simultaneously when newborns cried compared to when they did not cry ($Z = -2.903$, $p = 0.004$). Finally, Rectus Femoris was active alone more often when newborns cried ($Z = -2.411$, $p = 0.016$). This suggests a
pervasive level of activity of the Rectus Femoris during the stance phase in Experiment 2. Further, for both experiments and during swing and stance, the percentage when none of the muscles were active was higher when infants were not crying compared to when they were crying, but the differences were only significant in Experiment 2 ($Z = -2.132$, $p = 0.033$ during the swing and $Z = -2.551$, $p = 0.011$ during stance).

Figure 38: Muscle state for Experiment 1 for swing and stance. Significant differences are marked with *. 
Figure 39: Muscle state for Experiment 2 for swing and stance. Significant differences are marked with *.
7.4 Discussion

The overall aim of this analysis was to examine neuromuscular behaviour in stepping across two experiments where either treadmill speed or optic flow on a moving treadmill was manipulated. It was hypothesised that in general, muscular activation patterns would be less defined and predictable than those of older infants and show high levels of coactivation. The analysis also examined if proprioceptive-tactile or visual stimulation was able to modify the neuromuscular system and cause it to produce more adult-like patterns with less coactivation. Last, the role of arousal was investigated in more depth; an earlier analysis had shown that high arousal (crying) had made stepping more stereotypical (better swing range and smoother stance phase) and more coordinated (tighter) interlimb alternation. The current analysis looked for concomitants across neuromuscular strategies related to these changes. These may be observable in more defined probability profiles with higher likelihood of activation at appropriate times in the cycle, and possibly more reciprocal activation.

7.4.1 SECTION 1: Newborn EMG during stepping is variable and no ‘functional’ patterns for forward stepping are evident

The following section will discuss the findings on the overall characteristics of four leg muscles during stepping in newborn babies. These findings were descriptive only, and described example EMG signals from two newborns and POA profiles. Profiles were plotted for the example newborns, for the entire data set, for static and dynamic trials and for the comparable conditions between the first and second experiments.

7.4.1.1. Variability

The unpredictability of newborn muscle activation patterns during stepping was clearly observable in the example EMG plots during stepping on a static and a moving treadmill (Figure 30). Additionally, the POA plots of these same infants (Figures 29) illustrated that variability occurs intra-individually as well as between subjects. The POA profiles from the good stepper are quite flat, suggesting considerable variation between the EMG signal from this subject. Even the average stepper, who showed more defined profiles, did not show sharp peaks or troughs signifying the muscle was always active or always switched off. Differences for the same muscle between both subjects were also evident. When amalgamating POA profiles from all newborns, definition was decreased again,
confirming that all individuals contributed very different muscle activity patterns to the data pool.

In contrast, adult profiles show more defined profiles during walking cycles, with probability peaks of 100% in which the muscle is always active, and periods of time the muscle is always off (0%). Figure 41 illustrates this in the Tibialis Anterior, with Tibialis Anterior POA peaking during mid-swing and again at the initiation of stance. Similar peaks are observed at the start of the stance phase for the Biceps Femoris, towards the end of stance for the Lateral Gastrocnemius and during the beginning of the stance for the Rectus Femoris (see the adult profiles in Teulier et al., 2012). These peaks were not evident in the newborn EMG in this project. Teulier et al. (2012) also did not observe EMG predictability in infants across three age groups (one, six and 12 months) in most muscles, nor did the traces resemble adult profiles. Lateral Gastrocnemius was the exception: infants of all age groups showed similar POA size and shapes for this muscle, demonstrating ‘a pattern of probability approaching that of skilled adult walkers’ (p. 859). This project did not find an early precocity for the Lateral Gastrocnemius. However, the Tibialis Anterior muscle showed increased POA during the swing.

The data shows that newborn EMG responses are more variable than predictable. In contrast to Dominici et al. (2011), this study found no evidence for regularly occurring sinusoidal patterns during stepping. Although the shape of the wave-form was not directly assessed in the current study, multiple muscle bursts were possible during each step cycle,
with great variations in the timing and the relative amplitudes of these bursts. Even if nonnegative matrix-factorisation had been utilised to analyse the data, it is unlikely that two basic patterns could have accounted for 89% of the variance across the subjects in this group, even at different distributions or weights across the step cycle. The results from Teulier et al. (2012) contrasted Dominici on similar points. When these authors compared the results of their study to Dominici et al.’s, they proposed that the support surface may have played a role in the differences; Dominici et al. tested newborns in overground stepping whereas Teulier et al. tested them on a moving treadmill. However, the current study did not find consistency with Dominici for steps made on a static surface. This study supports the idea that the use of ensemble averages from steps from each child, which were subsequently averaged across the whole sample, may have masked the variability across step cycles.

When considering the gross movement outcome, it is not surprising that the muscle patterns are so variable: each step differed from the next in terms of leg movement trajectory and swing and stance duration. The source of this variability has been elegantly described by Bernstein (1967): there is a great number of options available in the neural system that can all produce the same outcome. These are needed to meet the needs of the movement outcome flexibly, in response to continuously changing forces and interferences. It is impossible for the newborn to have mastered the control of all these different degrees of freedom, particularly in the new environment they have just been introduced to. However, if the control structure was greatly simplified, for example, as proposed in the Central Pattern Generator theory, predictable patterns would be observable, particularly in a standardised environment as on the treadmill. With converse logic, the lack of patterned muscle activation in stepping suggests that even at birth, stepping must be more complex than hard-wired in the spinal cord and combined as building blocks to produce functional movement (Dominici et al., 2011). The evidence supports the idea of soft-assembly (Thelen and Ulrich, 1991), where the range of options provided by the system is freely combined until an efficient solution emerges under the influence of experience.

7.4.1.2. Functionality of muscle synergies during stepping

Additionally, the bursts were poorly coordinated in synergies that would be functional for forward stepping. This outcome was consistent with the findings in Teulier et al. (2012). These authors found that, although the kinematics of stepping stabilised across the first
year of age, neuromuscular patterns did not become significantly more adult-like during this time, excepting the Lateral Gastrocnemius, which showed reasonably advanced behaviour even during the first month. They proposed that although behavioural outcomes (i.e., the overt characteristics of stepping) may have become more consistent with experience, ‘the ways in which [infants] marshalled active and passive forces to produce the step pattern’ was decidedly unpredictable (p. 858).

However, Teulier et al. (2012) did find functionality on a more basic level: one-month-old infants showed more extension than flexion in stance and more flexion than extension during the swing phase while stepping on the treadmill. Dominici et al. (2011) also found increased extensor coactivity during stance and increased flexor coactivity during swing while stepping on a static surface, suggesting that newborns can direct control their muscle activity in a roughly efficient way. However, POA profiles in the current analysis did not provide evidence for such level of active control over flexion and extension in newborns who step on the moving treadmill or on a static surface. Newborns may have utilised muscles that were not measured, or employed unpredictable combinations of muscles to cause flexion and extension during steps on the static surface. On the moving treadmill, extension may have been passive. Alternatively, the lack of observable activity for flexion and extension may reflect a radical increase of variability associated with the transition from the womb to the outside world.

7.4.1.3. More defined likelihood of activation for the Tibialis Anterior

Only in the Tibialis Anterior, the current study showed some evidence for ‘functional’ muscle patterns. With a somewhat adult-like profile, Tibialis Anterior reached a POA peak of 85% during the swing phase in the static condition, suggesting that this muscle was active in 85% of steps during this time point in the swing. When stepping on a moving treadmill, the muscle was also more likely to be active during swing than stance, although the peak was attenuated to 60%. In adult walking cycles, there is predictable Tibialis Anterior activation during mid-swing and the start of the stance period to avoid catching of the toe during the swing and to initiate heel-strike. Interestingly, the Tibialis Anterior appears to be more adult-like at birth - albeit without showing a toe-lift at touch-down - and then become less predictable during infancy (Teulier et al., 2012, Forssberg, 1985). It may be that Tibialis Anterior activity has a purpose during the stepping in the womb, which is lost in the external environment and therefore not evident in the march-like steps that infants make during the first year of life. Mid-swing during tactile stepping
approximately coincides with the initiation of extension in air stepping. In the context of a confined uterine environment, lifting the toe may be more effective at the start of the extension phase. Alternatively, strong ankle flexion during mid swing may reflect the inability to dissociate ankle flexion from hip and knee flexion. Attenuating Tibialis Anterior activity during infancy may thus result from a growing ability to dissociate joint action with increasing experience, or simply lose its function outside the womb. The current experiment also showed that Tibialis Anterior activity during the start of the stance phase was attenuated in steps on the moving treadmill compared to the static condition. Perhaps the static condition is less novel and more like the uterine environment than the dynamic conditions. The unexpected movement of the surface below may trigger less predictable behaviour, or attenuated Tibialis Anterior activity in response to the novel environment.

7.4.1.4. Differences between static and dynamic surfaces

From the example EMG profiles (Figures 27 and 28), it seemed that muscles became more active on the dynamic surface compared to the static surface. This was not reflected in the POA analysis: muscles were not more likely to be active when the treadmill moved. For the Biceps Femoris, the Lateral Gastrocnemius and the Rectus Femoris, the probability that muscles were active did not exceed 62%. Only in the TA, the swing-time peak became more pronounced at approximately 85%, which approached adult values. The increased predictability of this muscle during times that adults also show peak activity suggests that treadmill movement may have made Tibialis Anterior activation more advanced. Alternatively, it is possible that the increased activation is associated with a different process, for example, the treadmill may have caused a rubbing sensation to the infants’ toes. Toe-lifting through excessive Tibialis Anterior contraction may have reflected a desire to keep the toes away from the treadmill belt. This, however, does not explain why this difference peaked during mid-swing until just after touch-down. Perhaps lifting the toe during this time is relevant inside the uterus, and the static environment may have allowed for natural behaviour to emerge more strongly than when the treadmill determined leg movement. For the remaining muscles, the moving treadmill did not make muscle behaviour more predictable, suggesting that even when the gross movement is constrained by the treadmill, variability is not observably reduced at the neuromuscular level.
7.4.1.5 Differences between the visually enhanced and non-enhanced treadmill

At first glance, the POA profiles in Experiment 2 appeared considerably more defined than those in Experiment 1 (Figure 30), with high activation during swing and low activation during stance for all optic flow conditions. This suggested that the response was more constrained in this experiment. Was this due to the lack of ability of optic flow to modify behaviour, or to a constraining effect of Dycem? To resolve this question, Figure 32 should be viewed. From these graphs, it is clear that the profiles from the comparable conditions are reasonably similar, except from the Lateral Gastrocnemius.

The Biceps Femoris and the Tibialis Anterior display the typical high activation in swing and lower activation during stance, whereas the Rectus Femoris shows a flatter profile. Differences were observed in the more predictable activation at the start of swing for all muscles - except the Biceps Femoris - in Experiment 2, which may reflect the desire of infants to pull away the foot from the strong stimulus that Dycem presents.

In contrast, the POA profile of the stance phase in the Lateral Gastrocnemius was different: this muscle was more predictably active during stance in the first experiment, supporting extension of the ankle during this phase. However, the likelihood that this muscle was active during stance did not exceed 0.6 and does not reach the peak values reached in Teulier et al. (2012) during mid-stance, which amounted to 0.8 in one-month-old infants, and nearly 0.9 in adults. Although optic flow and Dycem-enhanced treadmill changed muscle POA to a degree, the functional benefits were likely only minimal.

In general, the POA profiles were reasonably reproducible for the two comparable conditions across the two experiments. It seems that POA profiles for three of the four muscles in Experiment 2 roughly followed a shape that was typical for that muscle when stepping at slow treadmill speed. This suggests that Dycem did not cause the generic profile (high swing, low stance) apparent in Figure 30, but that the similarity of the POA profiles in Experiment 2 may reflect the limited effect of optic flow.

Although Dycem did not seem to cause the apparently more defined POA pattern in Experiment 2 there were some differences between the first and second experiment that are likely caused by the addition of Dycem to the treadmill belt. A secondary analysis showed that AR was consistently higher across all muscles in Experiment 2 (between the comparable conditions). An independent samples t-test found that these differences were significant for the Rectus Femoris ($t (36) = -2.515, p = 0.017$) and the Tibialis Anterior ($t (39 = -2.340, p = 0.025$), but not for the Biceps Femoris and the Lateral Gastrocnemius (Table 13). However, there were no consistent changes in burst length or coactivity.
between the comparable conditions in Experiment 1 and 2, suggesting that Dycem may have improved general muscle activity without having a systematic effect on the timing or predictability of muscle bursts.

Table 13: Activity ratio between Experiment 1 and Experiment 2, across the comparable conditions (slow treadmill speed and no optic flow).

<table>
<thead>
<tr>
<th>Activity ratio</th>
<th>T</th>
<th>Df</th>
<th>P-value</th>
<th>E1</th>
<th>E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biceps Femoris</td>
<td>-1.312</td>
<td>39</td>
<td>0.197</td>
<td>0.234</td>
<td>0.251</td>
</tr>
<tr>
<td>Lateral Gastrocnemius</td>
<td>-1.804</td>
<td>39</td>
<td>0.079</td>
<td>0.230</td>
<td>0.258</td>
</tr>
<tr>
<td>Rectus Femoris</td>
<td>-2.515</td>
<td>36</td>
<td><strong>0.017</strong></td>
<td>0.239</td>
<td>0.277</td>
</tr>
<tr>
<td>Tibialis Anterior</td>
<td>-2.340</td>
<td>39</td>
<td><strong>0.025</strong></td>
<td>0.256</td>
<td>0.288</td>
</tr>
</tbody>
</table>

7.4.1.6 Possible evidence for the increased activity at touch-down on the smooth belt

A note should be made on the apparent peaks around touch-down that were evident from subject 1 in the example data. There was a slight tendency for the POA of the Biceps Femoris, Lateral Gastrocnemius and Rectus Femoris to be higher around touchdown and toe-off in Experiment 1, particularly in the static condition, although the change was not great. Forssberg (1985) found that for ‘flexor and extensor muscles the activity often increased during transition from swing to stance and from stance to swing’ (p. 484). Forssberg attributed these ‘brisk peaks’ (p. 484) to reflex action triggered by ground contact. However, in the current study the peaks commenced before contact. As the time of contact was determined quite precisely in this study, the fact that the activity occurred before touch-down suggests that there may be a functional cause for the response. Okamoto (2001) also reported strong extensor activity before touch-down, but contrastingly, these authors did not see the phenomenon in one week old newborns but only from about one month of age. The authors attributed these extensor activity peaks to an early parachute response in the legs, which is described by Milani-Comparetti and Gidoni (1967) as the ‘reactions of the limbs to sudden replacements of the trunk’ (p. 633). This protective extension reaction is found in the lower limbs from approximately four months, laterally in the upper limbs at six months and forward at seven months. It is
peculiar that the current study found that a tendency towards increased muscle activity at touch-down was already observable at birth. The response may be a vestigial effect from the need for extra force at maximal extension in the intrauterine environment. It may be briefly suppressed by a change in context but returns when infants become habituated to the gravity environment (note that the infants in Okamoto’s study were studied longitudinally and may have been quite practiced at stepping by the time that the ‘parachute response’ appeared). However, it should be noted that the current study did not witness the activity peaks in all infants, and the slight increase in likelihood of activation may have been a reflection of overlap in burst timings alone. More research is needed to confirm a possible ‘parachute effect’ in newborns.

7.4.2 SECTION 2: Sensory stimulation affects EMG during stepping
In brief, sensory stimulation affected muscle behaviour, with different effects for tactile-proprioceptive and visual stimulation. Treadmill speed affected muscle responses somewhat unpredictably, with systematic results for muscle burst length only - bursts were significantly longer during static trials. There was no clear trend for POA and muscle state. For optic flow on the moving treadmill, the only significant differences were seen for POA, suggesting that faster optic flow tended to lower the likelihood of muscle activity, mainly during the stance phase.

7.4.2.1 Faster optic flow de-activated the hip extensor and ankle flexor in stance
The results for optic flow were unexpected. Across all muscles, there was a tendency for faster optic flow to lower the likelihood of muscle activity during the stance phase. In two of the muscles, Biceps Femoris and Tibialis Anterior, this difference was significant at the start of the stance phase. Additionally, the Rectus Femoris also showed a tendency for high POA in the congruent condition and low POA in the random condition during the start of the swing phase. The tendency of the Rectus Femoris to be more consistently active at the start of swing in the congruent condition may suggest that congruent optic flow promoted flexion during the start of the swing phase compared to random optic flow, although this effect was isolated and increased ‘functionality’ in response to congruent optic flow was not observed at any other time or in any other muscle. Additionally, the deactivation of the extensors during stance does not seem to serve a purpose. In adult POA profiles, the Biceps Femoris is activated consistently towards the end of the swing phase and reaches peak likelihood during the start of stance, where it has a propulsive
function. Increased extensor activity during stance was already reported in older infants (Teulier et al., 2012) and toddlers (Chang et al., 2006), but seems to be absent in this study. The Biceps Femoris was even less likely to be active when newborns were exposed to faster optic flow on the moving treadmill, decreasing the potential for an active push-off phase during stance.

However, the POA profile for the ankle flexor Tibialis Anterior was more adult-like, as adult POA profiles activate towards the middle and end of swing and deactivate during the beginning of the stance phase. Here, faster optic flow appeared to intensify this deactivation. Interestingly, the fact that the deactivation of the Tibialis Anterior occurred earlier in the swing phase with faster optic flow may suggest that this optic flow speed triggered the neuromuscular system into an earlier response. However, the effect was weak and not repeated in the other muscles. Overall, the results suggest that optic flow can modify stepping at the neuromuscular level however, the changes do not seem to universally advance muscle strategies to support adult-like stepping.

7.4.2.2 No systematic results for treadmill speed
The effects of treadmill speed on EMG were also subtle and not predictable. The Tibialis Anterior was more likely to be active on the fast treadmill but showed the lowest activation rates during the medium speed, suggesting no clear linear treadmill speed relationship for this muscle. The results from the muscle state analysis suggest that the Rectus Femoris was sensitive to treadmill speed, as the statistical differences were seen in combinations that included this muscle consistently. However, there was no clear direction to the effects. Strangely, the Biceps Femoris was least likely to be active during stance when the treadmill was moving slowly.

The lack of clear changes to neuromuscular strategy under the influence of treadmill speed suggests that the treadmill effect is mostly passive. The fact that the Biceps Femoris was less active during the extension or stance phase when stepping on the slow treadmill does not suggest that treadmill stimulation caused faster stepping through improved muscle activation. In that case, increased extensor activity would have been observed across the parameters. Increased step rate, or the improved step-to-pump ratio in response to treadmill movement (Siekerman et al., 2015) may just reflect the changed kinematics due to pull of the treadmill. The fact that the total number of movements increased may also be due to the fact that the duration of the movements was shorter, leaving more time for the initiation of new movements throughout the trial.
The effect of mechanical pull may explain the difference for muscle burst length between the static and dynamic treadmill speeds. The longest muscle bursts occurred when the treadmill was static and unaffected by movement from the belt. Perhaps muscle bursts were shorter because the legs were moved through their range of motion quicker. This also may have caused proprioceptive cues to be available sooner, thus increasing step rate. Unless these shorter muscle bursts were accompanied by significantly higher extensor amplitudes, shorter muscle bursts suggest de-activation rather than increased activity. These results suggest that the treadmill effect may be mostly mechanical and proprioceptive, without significantly changing muscular input at higher levels.

7.4.3 SECTION 3: The effects of arousal on EMG

Results from both Experiment 1 and 2 showed clear muscle activity increases for arousal. In the POA profiles, arousal appeared to raise the likelihood of activation in most muscles and across most phases of the cycle. The activity ratio (AR), which reflected the time a muscle was ‘on’ as a percentage of trial time, was significantly higher for all muscles in Experiment 1, and for the Biceps Femoris alone in Experiment 2 in response to arousal. For the muscle state analysis, arousal tended to increase the simultaneous activation between certain muscles, particularly those including the Rectus Femoris or the Biceps Femoris, however, no changes in coactivity during stepping was found.

7.4.3.1 Increased muscle activity with high arousal

Increased muscle activity levels (AR) were observed with high arousal in Experiment 1. At the same time, step and pump rate increased when newborns were more aroused. This suggests that arousal raised muscle activity so that more steps could be made although it is also possible that increased movement increased arousal reciprocally. However, the differences for AR were not significant in Experiment 2, except for the Biceps Femoris. Why were the results for these experiments so different? When comparing the AR results between the two experiments, the no OF condition in Experiment 2 showed higher AR levels across all four muscles compared to the slow treadmill speed in Experiment 1. This suggests that AR may have been generally higher in Experiment 2. Additionally, Chapter 5 noted that the number of steps was considerably higher in the second experiment compared to the first. The Dycem in the second experiment may have improved muscle activity and leg movements so strongly that any factors outside of this stimulus (i.e., optic flow or arousal) could not modify these parameters. The fact that arousal or optic flow
did not significantly increase stepping in Experiment 2 offers support for this idea. It is not clear why the Biceps Femoris showed significantly higher AR when crying in Experiment 2 when the other muscles did not. Perhaps the Dycem stimulus affected the Biceps Femoris less strongly, leaving more room for modification by external factors. Considering that Dycem enhances treadmill friction, and thus perhaps the mechanical pull effect, the hip flexors may be more constrained as they are extended during the stance phase on the augmented belt, masking any effects from arousal, whereas the Biceps Femoris may still be modified because it is not triggered by the increased treadmill pull. Indeed, when considering the AR of this muscle between the two experiments (Table 13), flexor activity increased significantly on the Dycem enhanced treadmill whereas extensors did not - with the Biceps Femoris showing the smallest inter-experiment difference. However, this does not explain why the Gastrocnemius did not behave the same as the Biceps Femoris in showing arousal-mediated increased activity in Experiment 2. Perhaps the factors and forces influencing lower leg activity are more complex due to their increased number of degrees of freedom: Gastrocnemius tension is also determined by knee and ankle movement.

However, it is also possible that the Biceps Femoris responded to arousal for an entirely different reason. Perhaps there is a link between the Biceps Femoris and arousal level, causing its effect to be stronger. Jamon et al. (2002) found that newborn rats showed a systemic extension response after L-dopa injection, causing ‘strong postural tonicity’ and extension of neck, trunk, tail and all four legs (p. 1016), and rhythmic activity after 10 to 15 minutes. L-dopa is converted to dopamine and noradrenaline in the brain. It is possible that aroused states cause a comparable raise in these neurotransmitters, activating the extensors and causing a slight activation bias for the larger leg extensors. In this case, arousal may have been strong enough to overcome the masking effect caused by the Dycem-enhanced treadmill in the Biceps Femoris alone.

7.4.3.2 The effects of arousal in newborns
The exciting effects of arousal were also described in an experiment on general movements in newborns (Hadders-Algra et al., 1993). These authors noted that the EMG of the upper arms in infants in behavioural state 5 (crying) showed higher movement amplitude and shorter between-burst intervals than when they were in state 4 (non-crying active state). General movements were more abrupt and vigorous. Additionally, this experiment found a decrease in muscle burst length with increasing arousal. Hadders-
Algra also found that the length of muscle activation decreased when arousal increased (between state 2) sleep and 4) active and non-crying). Increased muscle activation in response to arousal may be a result of lowered threshold and increased response amplitude, which is also seen in polysynaptic reflex responses of crying infants (Vecchierini-Blineau and Guihneuc, 1982).

The evolutionary benefits of increased muscle excitability with high arousal are clear, as they facilitate rapid and more forceful motor responses when an organism is at risk. However, Siekerman (2015) proposed that neuromuscular control may also change as arousal increases. Beuter and Duda (1985) suggested that in children, high arousal brings the execution of a task under more volitional control, making movements ‘less smooth and efficient’ (p. 240). Nevertheless, arousal must affect motor responses differently in adults and children. The motor behaviour described in Beuter and Duda’s study is under voluntary control, whereas the control of neonatal stepping is most likely spread across a much larger range of factors. In skilled movement, perception of stress and negative self-concept can interfere with its execution (Beuter and Duda, 1985). The relationship between motor performance and psychologically induced arousal is hypothesised to follow an inverted U shape (Neiss, 1988).

In newborns, response to arousal is likely to be more primitive. Improved efficiency and vigour of locomotor behaviour could play a role in survival and well-being in the uterine environment and in infancy. Sudden increases of vigorous foetal movement followed by no movement at all have been reported by some mothers after experiencing stillbirth (Linde et al., 2015). Perhaps this reflected a response aimed to remove the foetus from a position compromising oxygen supply (Sadovsky and Polishuk, 1977) or epileptogenic activity (Linde et al., 2015). The startle reflex seen with arousal from sleep after a hypoxic stimulus (McNamara et al., 1998) may also serve to protect young infants from suffocation.

Automaticity of movement in this context is not related to automatism in skilled movement. Skill ensues from fine-tuning of sensory-motor pathways under the influence of experience. Automatism in newborns may suggest a simplification of components responsible for the movement - or a freezing of degrees of freedom, in order to maximise motor output.
7.4.3.3 Can arousal change muscle activation patterns during stepping?

Therefore, the following question is of interest: can arousal selectively alter muscle activity - suggesting a shift in control? The current experiment showed that the proximal muscles were more affected by arousal in both experiments. Rectus and Biceps Femoris were more likely to be active when arousal was high in the POA analysis, whereas there were no pervasive differences for Tibialis Anterior and Lateral Gastrocnemius between crying and non-crying steps. Conversely, Beuter and Duda found that in a stepping task with children, proximal joint behaviour was not affected by arousal whereas the distal joints became more variable as a function of arousal (1985). Again, it is necessary to consider the stage of motor development. The children in the task were asked to step over obstacles at different levels of arousal. The authors suggested that ‘distal joints may be more susceptible to higher order processing’. Neonates are not likely to have progressed to this level of control yet, and the explanation for the sensitivity to arousal level must be found elsewhere. From a contextual perspective, increased hip flexor and extensor sensitivity response to arousal might be a functional feature, as these are the muscles that are most likely to cause gross movement inside the womb. Alternatively, the control of the limbs is thought to be cephalocaudal, particularly in the development of balance (Woollacott and Shumway-Cook, 1990) and proximal to distal in reaching (Berthier et al., 1999). Accordingly, Hadders-Algra noted a difference in the upper arm muscle vigour and not in the fore-arm (Hadders-Algra et al., 1993).

7.4.3.4 No evidence for increased muscle efficiency or patterned stepping with high arousal

There seems to be little increased reciprocal activation for the muscles during high-arousal stepping, although in Experiment 1, swing appropriate synergists Rectus Femoris and Tibialis Anterior did activate simultaneously significantly more when crying compared to non-crying steps. POA profiles did not show more ‘mature’ or functional movement with higher arousal, and there was no change in step coactivity during crying. No obvious increase in synergist activity in response to crying was evident from the muscle state analysis. This is consistent with the findings of Hadders-Algra and her colleagues, who did not observe arousal-mediated differences in coactivity (Hadders-Algra et al., 1993). In the current study, General Coactivity (GC) was lower during non-crying. As GC represents coactivity during the whole trial, including non-stepping periods, it also includes coactivity during random movements or pumps. It is possible that
the increase in coactivity may be mostly limited to pumps, although this can only be confirmed through an assessment of coactivity during pumps alone. Alternatively, it could reflect an increase of general tonic activity.

Yang et al. (1998a) reported that infants were able to produce reciprocal activation regardless of age, and interpreted this as a sign of functional muscle patterning for stepping. From this point of view, the lack of change in coactivity or synergist activity does not support the idea that control shifts towards more spinal control with high arousal. Even when disregarding the idea that spinal patterns control stepping at birth, high arousal or not, there are no signs that crying improves the ‘efficiency’ of forward stepping. Arousal may have a generic stimulating effect on muscle activity, without advancing or facilitating functional muscle strategies. Yet, it seems very capable of improving overall muscle output and increasing stepping.

7.4.3.5 Differences in arousal response with different sensory stimuli

Interestingly, arousal affected muscle behaviour differently when infants were exposed to optic flow and Dycem, compared to being on a moving treadmill at different speeds. The differences in arousal effects between the two experiments may just be a result of having two different samples of infants. However, it is very likely that sensory modality altered the way arousal affected EMG. The differences between experiments for AR have already been described in the first paragraph of Section 3. Additionally, General Coactivity increased for two muscle pairs as a function of arousal in Experiment 1 but not in Experiment 2. However, muscle burst length only changed in response to arousal in Experiment 2. Different muscle combinations showed simultaneous activation between the two experiments. Only POA showed a similar trend of increased likelihood of activation for arousal, but across completely differently shaped profiles.

The fact that AR increased from the first to the second experiments when looking at similar conditions (no OF and slow treadmill speed in both experiments) suggests that the treadmill belt covering – the major difference between the two experiments – had an activating effect on the neuromuscular system. The fact that general AR levels were higher may have masked some other effects that optic flow may have had. Although it is not possible to compare directly between experiments, the data seems to suggest that on the moving treadmill, arousal affected step rate and activation ratio more freely but on the Dycem augmented treadmill and with optic flow, muscle activation during steps was determined mostly by the Dycem.
7.5 Limitations

There are some limitations that need to be mentioned. The traditional method of calculating coactivity between muscles had very small sample numbers due to poor recording of some muscles and drop-out of subjects due to the fact some newborns did not make steps while they were in both behavioural states. Low sample size considerably reduced power in this study. Although it is not possible to calculate post hoc power for the Friedman analyses, the results when calculated parametrically and nonparametrically were sufficiently similar that post hoc power was approximated through a parametric analysis of the data. Here was shown that for the analysis with the smallest n-number (the Rectus Femoris in E13, n = 7), power in the POA analysis was 0.2341. Assuming equal power between parametric and nonparametric analyses when used on appropriate data, sample size needed to be increased to at least 24 to ensure a power of 0.8. Due to the differences between the two studies, it was impossible to amalgamate the results in the arousal analysis to increase sample size. Ideally, this study should be repeated with at least 72 subjects, or drastically improve EMG data capture methods, to ensure a full data set for all muscles in 24 infants. The scope of this investigation would be sufficient for a new PhD or postdoctoral project.

Although it is likely that significant differences were missed in the EMG analysis of this project, trends in the data showed where possible effects occurred. Even if the strength of these effects cannot be determined, the results from this exploratory study can be used to fill knowledge gaps – for example, what newborn EMG looks like during stepping, and if they respond to sensory stimuli - and indicate where the focal points of consecutive research may lie.

7.6 Conclusion

The current study confirms that motor patterns are considerably variable in newborns, extending the current knowledge base to treadmill stepping in infants within three days of birth. Although sensory stimulation affects muscle activation, effects are unpredictable for tactile-proprioceptive stimulation from the treadmill. Optic flow affected EMG in stepping less strongly, possibly because the responses were determined by the Dycem-enhanced treadmill, although faster optic flow systematically deactivated the muscles
during the stance phase. Arousal played a facilitating role by enhancing muscle activation – without advancing muscular patterns towards more efficiency or automatism.
Chapter 8:
GENERAL DISCUSSION
8.1 Introduction

The aim of this thesis was to examine whether newborns would tolerate stepping on the treadmill and if they were able to utilise treadmill movement to improve their step rate, stepping quality and coordination. Further, it aimed to investigate the effects of treadmill speed and optic flow condition on a moving treadmill on stepping behaviour, kinematics and muscular activation strategies. Arousal level was recorded to ensure newborns tolerated the testing and to determine which subjects needed to be excluded due to excessively high or low arousal levels. Continuous monitoring of arousal also offered the opportunity to directly map arousal onto steps, and examine arousal effect on stepping quantity, quality, kinematic parameters and muscle behaviour. The key findings from the two research studies performed can be summarised as:

- Newborn babies responded to treadmill movement with more leg movements. These movements could be divided into pumping in place and stepping forward. Treadmill movement improved only forward steps and not in-place pumps.
- Newborns did not adapt to treadmill speed as strongly as their older peers did, although treadmill movement was able to modify behaviour. Differences in cycle duration, stance ratio and muscle burst length occurred between the moving treadmill conditions and the static condition. The response of neuromuscular behaviour to different treadmill speeds was variable and did not indicate a general effect.
- Optic flow on the treadmill did not affect step rate, quality or coordination. There were more subtle effects to joint angles and intralimb coordination, with less synchrony and less hip extension when optic flow was congruent to the treadmill. Additionally, faster optic flow tended to deactivate most muscles during stance. The advantage of adding optic flow appears to be small in this project.
- Arousal had a stimulating effect on leg movements; when on the smooth treadmill alone, all movements increased when infants were crying and on the augmented treadmill, pumping increased. Additionally, crying infants made ‘better’, further forwards placed steps with smoother stance phases. Improved extension was seen in steps made while highly aroused. Additionally, arousal was associated with tighter alternation between limbs and increased between-joint synchronicity. The EMG analysis did not show more effective neuromuscular strategies but found increased likelihood of activation for proximal muscles with high arousal. The increase in step
rate, improved quality and tighter coordinative forms may be a result of an overall gain in muscle energy.

- The results offer further support for a dynamical systems view of stepping through the evidence of great variability in step rate response, kinematics and muscle behaviour.

The main discussion will go through these key findings in detail. It will also describe an unexpected observation: Dycem appeared to strengthen the tactile stimulus and/or augmented mechanical pull and may have masked certain optic flow effects. The discussion will then also describe how the findings link newborn stepping to intrauterine leg movements. Finally, the limitations and future work will be described, followed by a conclusion.

8.2 Treadmill motion improves forward stepping at birth

A moving treadmill is able to increase stepping in infants as young as three days of age. Interestingly, treadmill movement improved only one type of flexion-extension cycle; more forward steps (with potential for locomotion in the upright position) were made, whereas the number of vertical cycles (pumps) remained the same. The apparent improvement in advanced behaviour may justify bringing forward the start of treadmill training interventions. However, it is essential that treadmill training targets the appropriate pathways in stepping: training should optimise the volume of perception-action cycles but also incorporate a variety of appropriate stimuli as it needs to engage ‘many sensory systems in coupling with the motor system’ (Teulier et al., 2015, p. 453). The training environment should not constrain the steps too strongly - as would be evident from a lack of variability in the gross motor and/or neuromuscular output - because this may limit the later availability of multiple neuromotor pathways. On the treadmill, this may involve stepping at different speeds or in different directions, on smooth and frictional surfaces, and with modifications of individual constraints like leg length or weight (Teulier et al., 2013). Additionally, contextually appropriate stimuli that span the nervous system and mimic the natural environment - for example, visual, olfactory, auditory and perhaps vestibular stimuli - will put stepping into a functional context at an early age. This may give them an early opportunity attune to these stimuli, bringing forward critical abilities to allow for independent walking to emerge.
It is not likely that training stepping from birth perpetuates reflex action before cortical pathways are available – as was first proposed by Thelen and Fisher (1982). Dynamical systems theory poses that it is unlikely that stepping is merely a reflex or centrally mediated pattern (Teulier et al., 2015, Thelen and Ulrich, 1991). A recent study by Kanazawa (2014) provided evidence for this idea by showing that newborns, and even premature babies from 36 weeks of gestation, show descending corticomuscular coherence in supine kicking right from birth. It is likely that stepping, with kinematics very similar to kicking (Thelen and Fisher, 1982), also is under the influence of the primary motor cortex, particularly because cortical neuromotor pathways are available and functional in utero (Eyre et al., 2000) and only require attuning and pruning to become more effective. This study further examined the evidence for reflex action and central pattern generation in stepping by examining effects of sensory stimulation, particularly by how the different types of sensory stimulation affect quantifiable measures in stepping. The level in which different modifications occurred may be of theoretical interest.

8.2.1 There was no adaptation to different speeds

Although treadmill movement alone was able to modify behaviour, newborns did not seem to be highly sensitive to different speeds yet; there was no significant linear relationship between treadmill speed and step rate. Step rate tended to respond to increasing speed with an inverted U curve, with a decreasing step rate for the fastest treadmill speed. It may be that treadmill speeds around 0.172 m • sec⁻¹ were optimal for the majority of newborns in this experiment. There were no further meaningful differences between speeds, but treadmill movement in itself modified step coordination and cycle analysis regardless of its speed: it improved parallel stepping, shortened the step cycle duration and decreased the time spent at stance. Additionally, treadmill movement tended to shorten muscle burst lengths. All the changes between the static and moving treadmill suggests that at the speeds offered in this project, treadmill movement externally supplied the energy for stepping. This afforded more bilateral leg movement, increased the number of steps, and mechanically determined the length of the cycle through the timing of the stance phase. This confirms the role of proprioception in stepping. Furthermore, it suggests that energetic demands are an important limiting factor in newborn stepping, which can be facilitated by treadmill movement.
8.2.2 Treadmill stimulation affects stepping through timing of step cycles

The lack of direction in the POA and muscle state results suggests that newborns’ responses to different treadmill speeds were highly variable. Even between static and dynamic trials, the results did not systematically show a trend, with the exception of muscle burst length across the whole trial: muscle bursts on the static treadmill were longer than those on the dynamic treadmill. This may be explained by mechanical pull: the treadmill may have aided the muscles during the stance phase, shortening the time of their contraction. As the hip moved from flexion to extension, proprioceptive cues would have become available to flex the hip and commence a new swing phase on the dynamic treadmill belt, whereas in the static situation, these cues would have been limited. The treadmill experiments by the Edmonton group (Yang et al., 1998a, Yang et al., 2005, Pang and Yang, 2002, Pang and Yang, 2000, Lam and Yang, 2000) attributed infants’ adaptability to direction and speed when stepping on the treadmill to sensitivity of the central pattern generator to sensory cues. Passing a certain degree of hip stretch is thought to trigger flexion in any direction, although most reliably in the forward direction.

Hip flexion in response to extension may be a spinally mediated sensory loop even without the presence of a central pattern generator. However, there may be a different explanation for the extension-flexion response in newborns. The hyper-flexed posture of newborns while stepping is well-described (Thelen et al., 1984, Forssberg, 1985, Teulier et al., 2015). Newborns are accustomed to being tightly flexed inside the womb due to space constrictions (Piontelli, 2014), and hip flexion in response of extension may reflect a resistance against a completely novel position of the leg. Moreover, it is not clear whether infants directly after birth are sensitive to maintaining a base of support underneath their centre of mass, but this could certainly play a part in treadmill stepping in older infants. Teulier et al. (2012) noted that it was not clear if infants actually aimed to step, or tried to overcome a sense of instability from the moving surface below.

The lack of effect for different speeds shows newborns were not sensitive enough to match treadmill speed more closely, which contrasted results from a study across infants from a wider age group (Yang et al., 1998a) where speed and cycle duration were closely related. The effect of the treadmill on stepping seemed to be limited to the rough timing of flexion and extension through sensory cues, without causing differences in the interplay between muscles. This lack of fine-tuning may reflect the intrinsic abundance of variability typical to any system in transition, but also that stepping is influenced by multiple factors in addition to proprioceptive feedback from the hip. Thus, the
unpredictability of the stepping responses (Thelen, 2008, Thelen and Ulrich, 1991) and highly variable neuromuscular patterns (Teulier et al., 2012, Chang et al., 2006) in the current study offer further support to the dynamical systems view of stepping and its development.

8.2.3 Dycem strengthens the tactile-proprioceptive effect of the treadmill

Additional support for the role of the treadmill in muscle burst timing may be found in the fact that Dycem seemed to strongly increase step rate in comparison with the smooth belt - without increasing arousal: there was no significant difference in arousal between the slow treadmill condition in Experiment 1 (mean 3.96) and the no OF condition in Experiment 2 (mean 4.10; \( t(38) = 0.464, p = 0.646 \)). Dycem does not allow for sliding of the foot across the treadmill, and enhances the pulling effect of the treadmill. This may have increased step rate between the two experiments simply because the foot did not have the opportunity to remain in a stationary position on the treadmill. The high-friction belt covering masked the effects of optic flow and arousal on the treadmill insofar that it showed only changes for optic flow when the foot was minimally in contact with the treadmill belt – i.e., for pumps – or when the behaviour had not already reached its maximal rate. Some subtle changes were visible at the kinematic and neuromuscular level; however, end-effector behaviour was strongly determined by the treadmill.

8.3 Optic flow affects stepping but the effect may be attenuated by treadmill movement

Optic flow affected forward stepping only on a subtle level in this project. No changes were observed for rate, quality or coordination, although the number of pumps per second changed. Pumps occurred most when the optic flow was random. Maximum hip joint angle also increased for random optic flow compared to congruent optic flow. Hip-knee joint synchronicity increased for random optic flow compared to congruent optic flow. Finally, faster optic flow tended to deactivate most muscles at the start of the stance phase, but became significant in the Biceps Femoris and the Tibialis Anterior only. An explanation for the fact that optic flow did not have more pervasive influences is proposed in the previous paragraphs; the treadmill, especially when covered with Dycem, may have determined step rate and timing mechanically. However, despite this strong modifier, effects could be observed in the behaviour of the less constrained joints and of the neuromuscular system. The following paragraphs focus in on these results in detail.
8.3.1 Congruent optic flow decreased joint movement synchrony

High levels of synchronicity between joint movements are typical for infant and newborn stepping. Thelen and Cooke showed that hip-knee synchronicity only decreased at a very late stage in the development of stepping: after the attainment of independent walking (Thelen and Cooke, 1987). The current project found that optic flow was able to affect the lag between peak flexion rates in the hip and knee. Compared to random optic flow, congruent optic flow showed more decoupling, or greater lag. This may be related to the fact that there were significantly more pumps in the random condition; pumping is characterised by simultaneous joint flexion and extension.

However, it may also reflect an early sensitivity to congruent optic flow. There was a nonsignificant difference for inter-joint coupling (percentage lag, see Table 6) between the random optic flow and the no optic flow condition (no OF), with the percentage lag for no OF approaching the percentage found when optic flow was congruent (approximately 16%). Similarly, there was a nonsignificant difference between the congruent and the faster optic flow, with percentage lag for the faster optic flow reasonably close to that of the random optic flow (6.3 and 4.3 respectively). Even though differences were nonsignificant after correction of multiple comparisons, their p-values were below 0.05. When considering that some do not support correction for multiple comparisons in exploratory studies (Sinclair et al., 2013), these additional results show further support for the tendency for joints to decouple under congruent or non-conflicting conditions.

A sensitivity to the coherence of optic flow was shown in a study by Barbu-Roth et al. (2016). The optic flow was identical to that utilised in this project and when newborns were exposed to incoherent (random) optic flow, step lengths were shorter compared to when they were exposed to congruent optic flow (moving toward or away from the infant). These short steps may be similar to the pump-like behaviour seen with random optic flow in the current project.

The difference between the aforementioned study and the current project is that treadmill movement was added, however, sensitivity to coherence seems to be the common theme. Perhaps newborns stepped more naturally in the conditions without conflict within the stimulus or between stimuli. In the conditions where the optic flow moved differently to the treadmill, infants may have aimed to pull the feet away from the treadmill to an extent. However, at the same time, peak extension was greater during random optic flow, suggesting that there was no decrease in stance phase length or weight acceptance when
optic flow contradicted treadmill movement. Perhaps these results reflected an increase in stereotypical stepping and movement vigour caused by conflicting information without a concurrent increase in arousal. The effect that random optic flow appeared to have on stepping in the current context is not entirely clear, but the results suggest that congruent optic flow may offer an environment which allows more variation and exploration.

8.3.2 Faster optic flow allowed for a more passive stance phase
Although only significant for the Biceps Femoris and the Tibialis Anterior, the POA profiles of all muscles showed a decrease in activation probability at the beginning of the stance phase in response to faster optic flow. This muscles strategy of high activation during the swing phase and low activation during the stance phase is only efficient for walking for the Tibialis Anterior, and not for the Biceps Femoris (hip extensor/knee flexor). The faster optic flow appeared to intensify, and perhaps ‘speed up’ the general muscle response adopted by newborns in this context. The treadmill affects the temporal aspects of stepping through the interplay between mechanically induced extension, triggering flexion, and pre-loading of the stance leg, enabling forward swing (Thelen, 1986b, p. 1503). However, the role of optic flow may be more complex.

The fact that faster optic flow was able to modify muscle behaviour in the presence of a Dycem enhanced treadmill was surprising, as it suggests that sensitivity to optic flow speed is able to overcome this influence on a neuromuscular level. However, newborns were likely not strong enough to overcome the treadmill to generate changes to the overall execution of the movement with these neural modifications. Visuo-postural responses are proposed to act through indirect neural tracks and depend more on a precocious ability to match optic flow speed to postural movement than on speed recognition (Jouen et al., 2000). The task of stepping is more complex than postural responses, and therefore the optic flow effect may be considerably more variable. Although sensitivity to optic flow speed is thought to become rapidly more efficient from approximately three months of age (Aslin and Shea, 1990, Bertenthal and Bradbury, 1992), it is possible that basic functional speed sensitive links are available for stepping from birth. These links merely require experience to become efficient. Moreover, this suggests that neonatal stepping is affected by many factors rather than a sensory mediated CPG alone. These factors influence stepping in various ways, and depend strongly on the way in which sensory stimuli are presented.
8.3.3 Does optic flow on the treadmill offer the same benefits as it does in older infants?

Previous research has shown that congruent optic flow on the treadmill enhances the sensory environment, causing increased stepping and step complexity (alternated stepping) in typically developing infants from two months of age (Moerchen and Saeed, 2012). It also increased step frequency in infants with spina bifida between two and 10 months old (Pantall et al., 2011), although the effects were stronger for older infants. Additionally, optic flow activated muscle activity in infants with spina bifida (same age group), although this only translated into more steps for the oldest infants (Pantall et al., 2012).

Conversely, the experiments in this thesis did not find improvements in muscular activity, coordination or stepping as a result of added congruent optic flow on the treadmill. Interestingly, Pantall et al. (2012) found that friction and congruent visual flow conditions produced similar neuromuscular effects. Therefore, Dycem may have masked some of the optic flow effects on muscle patterns in Experiment 2. It is difficult to predict how optic flow might affect stepping on a treadmill belt that is not augmented with Dycem. However, research suggests that optic flow appears to affect older infants more strongly, particularly in infants with spina bifida. The visuo-motor link develops under the influence of experience; therefore, it is possible that infants at birth only show a weak response to coupled visual and tactile-proprioceptive stimuli. Further research is needed to further examine the possible benefits of optic flow on newborn treadmill stepping, for example, in studies with a weaker tactile-proprioceptive component. For instance, in a study with newborns stepping on a smooth treadmill belt, or on a treadmill running at a slower speed, the optic flow effects shown in air-stepping (Barbu-Roth et al., 2009) may become more observable. Alternatively, a longitudinal study testing newborns every week until the age of two months could examine what age or experience level is required for optic flow to be observed next to the treadmill stimulus.

8.4 Arousal increased step and pump rate, altered step kinematics and increased muscle activity

Overall, high arousal increased stepping and pumping when on the treadmill, although the effect was significant for pumping alone - and not stepping - on the treadmill augmented with optic flow and Dycem. Step quality and interlimb coordination became more advanced on the smooth belt, and intralimb coordination became more synchronous
on the augmented treadmill. High arousal was also associated with increased hip extension and knee range of motion. Overall muscle activity increased on the smooth treadmill, but only the Biceps Femoris was significantly more active with high arousal during the optic flow-Dycem experiment. Across the whole trial, muscle burst length decreased for one muscle on the augmented treadmill, and general coactivity increased for two muscle pairs on the smooth treadmill. During stepping, the two proximal muscles were more likely to be active during high arousal. No changes occurred for coactivity during steps, but muscle state analysis revealed a tendency for increased activity of muscle combinations that included the Rectus Femoris or Biceps Femoris with high arousal. Interestingly, an increased percentage of the “all muscles off” combination occurred for low arousal, but only during the optic flow-Dycem experiment.

8.4.1 Arousal: more stepping and more muscle activity
Movement rate increased when arousal was high, when infants were crying. The stimulating effect of arousal in infants and newborns was earlier seen for reflexes by Vecchierini-Blineau and Guihneuc (1982), for general movements by Hadders-Algra (1993) and for latency of stepping by Domellöf (2007). Thelen also noted that it infants needed to be at higher aroused states in order to make gross motor behaviours such as kicking or stepping (Thelen and Fisher, 1982), which led to scientists attempting ‘to excite the infant through play’ in order to elicit more steps (Musselman and Yang, 2007, p. 1248). Thelen proposed that the increased ability to lift the legs due to generic excitation aids stepping (Thelen, 1986b). Support for this was found in the fact that particularly the Biceps Femoris and the Rectus Femoris, the two proximal muscles most effective at causing flexion and extension, were more likely to be active when newborns were crying whereas there was no change for the distal muscles. The proximal muscles were also most likely to be coactive with other muscles in the step coactivity analysis and the muscle state analysis.

It is possible that stepping increased with higher arousal because the muscles were more readily active and able to counteract gravity and support weight. However, high arousal was associated with more advanced step quality and coordination in the first experiment. It was proposed that arousal made stepping more automated and stereotypical (Siekerman et al., 2015); perhaps by shifting the regulation of stepping towards spinal control. Alternatively, it was possible that arousal made movement more efficient without
activating any pattern generators. To confirm this suggestion, joint behaviour and muscle strategies needed to be examined in more detail.

8.4.2 Extension increased with high arousal

Note that 3D kinematic analysis was only performed for Experiment 2; it is not possible to generalise effects seen in Experiment 2 across to Experiment 1. In Experiment 2, on the moving treadmill enhanced with Dycem and optic flow, arousal was associated with increases in hip extension and extension rate. Improved overall muscle energy may have caused newborns to push their leg out more strongly in extension, allowing them to accept more weight on the stance leg. Putting weight on the leg causes the foot to move more smoothly with the treadmill belt, improving the stance phase and allowing newborns to reach a larger hip extension angle. The fact that hip extension rate was significantly increased during the stance phase of the steps supports this idea.

The fact that angular velocities were only significantly different for extension and not flexion seems to suggest that the extension response was stronger than the flexion response. Although this may be possible, as strong leg extension was reported in newborn rats after L-dopa injection (Jamon et al., 2002), it is also important to consider that hip extension rate and maximal angle may have been determined by a combination of treadmill speed and the baby’s strength to weight ratio. When extending the leg on the treadmill, one has to contend with the fact that the foot cannot move back faster than the treadmill allows. In response, the body has to go upward, which is constrained by the newborn’s weight bearing capacity. The hip might move more freely in flexion, causing more variability. The fact that maximal angular velocity of the hip was also higher with high arousal, but failed to reach the significant level at a p-value of 0.074, supports this idea. The analyses of muscle activity do not support hyperactivity of the extensors, which suggests that the latter explanation is the more likely one.

8.4.3 Improved joint velocity synchronicity with high arousal

Additional to the apparent extension bias, kinematic analysis showed increased synchronicity between maximal hip and knee extension rate with high arousal. In adult walking, overall maximal knee extension rate occurs at a different time of the step cycle (Umberger and Martin, 2007). Increased synchronicity may reflect an increased inability to decouple joint actions with high arousal in newborns; high arousal may have increased their tendency to freeze degrees of freedom in step organisation. However, simultaneous
fast hip and knee extension is also associated with marching (Thelen, 1986b). It was observed that pump rate improved under the influence of arousal in the Dycem-optic flow experiment, which may also have been reflected in an increase of joint synchronicity, specifically because the kinematic analysis on joint behaviour on the whole cycle includes data for both steps and pumps. The fact that only pumps improved under the influence of arousal was earlier explained with the idea that step rate was more strongly constrained by Dycem than pumping.

8.4.4 The effects of arousal on coactivity

Babies show considerable coactivation between synergists in stepping throughout infancy (Okamoto and Okamoto, 2001, Teulier et al., 2012). Even during walking in toddlerhood, neuromuscular strategies have not yet been optimised completely (Chang et al., 2006). Coactivity, or co-contraction, is thought to ‘reflect infant brain development in response to early attempts to achieve a goal’ (Teulier et al., 2015, p. 450). With experience, responses become more specific and attuned. Consistently, Okamoto et al. observed that reciprocal activity improved slightly as a result of experience (2003, 2001). However, Yang already saw considerable reciprocal activity across all ages (from 10 days to 10 months of age), which was attributed to the existence of a functional CPG for stepping in this study.

When considering the idea offered in Siekerman et al. (2015) - that better step quality and coordination in response to high arousal may be caused by a shift towards more automated, spinal control – one may expect improvements of reciprocal activity during steps made while crying. Conversely, this project observed increased coactivity of traditional antagonists with high arousal, but on the smooth treadmill only (Experiment 1), and only when measured during the entire trial. There were no differences between coactivity when measured during steps alone, or for any coactivity measures in Experiment 2. Overall, coactivation was high, and consistent with coactivity values in Teulier et al. (2012), who measured coactivity in infants between one and 12 months. Coactivity did not decrease as a function of crying in the current project, showing that muscles did not tend to adopt more reciprocal patterns with high arousal. This finding is consistent with Hadders-Algra et al. (1993), who found no arousal-related changes for antagonist or synergist coactivity in the arm muscles of newborns during spontaneous movement. As the only study reporting the finding of reciprocal activity, the results from Yang et al. (1998a) are not convincing: they poorly quantified their reciprocal activity
measures and did not report coactivity by age group, despite their claims that clear reciprocal activity was found for all ages. It is possible that their observations occurred in infants who had received considerable training, and therefore they may not have been a reflection of pattern generation but instead, shown experience mediated adaptation and attuning of muscle patterns. It may therefore not be rational to expect decreased coactivity and increased reciprocal activity in response to increased arousal, even if the control of stepping did shift towards a more automated, spinal drive. More compellingly, the coactivity analysis was characterised by the same high level of variability that was typical for the majority of parameters measured in the current project. The muscle strategies utilised for making steps at birth are variable in any behavioural state, crying or non-crying, suggesting that muscle strategies are not determined by one simple mechanism such as proposed by CPG theory.

8.4.5 Arousal is not associated with more efficient muscle strategies

Instead, newborns and infants produce highly variable muscle strategies, and there may have been modifications to less conventional muscle combinations than those included in the traditional coactivity analysis. The muscle state analysis considers all possible muscle combinations and is therefore a more appropriate method for early movement development. In the Experiment 1, on the smooth belt, arousal improved the simultaneous activation of the Rectus Femoris and the Tibialis Anterior during swing. Although no synergists, these muscles do play a role during the swing phase, as they cause lifting of knee and toe to allow the foot clear the ground. During stance, high arousal had a slight attenuating effect on the Tibialis Anterior-Biceps Femoris-Lateral Gastrocnemius combination. These muscles work together to flex the knee but counter each other in plantar and dorsiflexion of the ankle; though decreasing its coactivity may decrease knee flexion activity during stance, the magnitude of the effect was small and probably negligible within the synergy between all the different leg muscles.

In Experiment 2, crying improved the simultaneous activation between Biceps Femoris and Tibialis Anterior during the swing phase. These muscles are not synergists or antagonists. As the Biceps Femoris is also a weak knee flexor utilised at the initiation of swing, the muscles may indicate improved muscle strategy for the beginning of this phase, just like the co-activation of the Rectus Femoris and Tibialis Anterior did in Experiment 1. Conversely, the Rectus Femoris was activated on its own for a considerable portion of the stance phase. Ideally, the Rectus Femoris is not active, particularly during
the start of the stance phase, as it hinders extension of the hip. Additionally, there was some evidence for the Biceps Femoris and Rectus Femoris to be activated in concert with high arousal during the stance phase, although the difference with non-crying states was small. However, the difference was greater when the Lateral Gastrocnemius was added to the pair. This combination of muscles during stance suggests a tendency to lock the leg at the hip, and may reflect an attempt to stabilise the stance leg. All in all, the effects of crying on functional muscle strategies were variable and did not suggest a consistent improvement of efficiency during the swing and the stance. However, muscular synergies depend on more than temporal analysis; the amplitude of the muscle contractions were not analysed in this project. Additionally, muscle state was computed across the entire swing and stance phases, possibly masking more specific responses at significant events in the step cycle. Furthermore, many other muscles are also active to produce movement (Teulier et al., 2012, Sansom et al., 2013), and it is possible that the current analysis was too superficial to capture the activity of all muscles utilised in the step. However, the results highlight that muscle behaviour is excessively complex and even though small improvements of efficiency may have been masked, muscular behaviour in newborn stepping is decidedly variable.

8.4.6 Did variability change in response to high arousal?

Even if muscle behaviour did not become more reciprocal with high arousal, it is possible that support for an arousal-mediated shift towards automated control was evident within the variability of the muscle bursts. If pattern generation was caused by increasing arousal, variability in the muscle responses would likely decrease. Dominici et al. (2011) found that ‘two sinusoidal-like patterns’ accounted for nearly 90% of the variance across newborns (p. 997), suggesting that muscle activation strategies are very predictable across cycles. However, variance was computed over averaged cycles and did not quantify inter-cycle variability. In this thesis, variability of neuromuscular patterns was measured within the POA analysis, which takes into account variability across cycles and individuals. Although some muscles were more likely to be active across the entire step cycle, POA profiles did not become more defined when infants were crying, suggesting that the predictability of muscle contraction during appropriate points in the cycle did not increase. No specific activation patterns were found for any of the arousal levels, confirming the previous findings that high arousal did not bring stepping under control of a CPG.
8.4.7 Can improved hip extension with high arousal be explained by increased muscle energy alone?

Although the ability to extend the hips more and faster with higher arousal may point towards better muscle efficiency around the hip, the analysis of muscle behaviour failed to support this concept. Even though the Biceps Femoris alone showed more overall activity with high arousal in Experiment 2, this was not measured during steps alone and is not strong enough evidence in itself. It is possible that overall increased muscle readiness and strength caused the changes seen with arousal. This was reflected in the improvement of the flexion rate and ROM of the knee. Additionally, it must be noted that there was a tendency for more universal improvement of flexion and extension rates, although these did not reach significance – nevertheless, some were quite close (see Table 6). They may not have reached significance because certain joint movements may have been more variable than others. Particularly the extension rate of the hip may have been constrained more strongly (by the treadmill belt and the infants’ ability to bear weight on the leg), giving more predictable extension rates. Hip flexion rate may have been more variable, because the leg moves freely into the air. This idea is supported in the fact that the differences between flexion rates appeared to be much greater than between extension rates, but were only predictable enough in the knee to reach significance. Overall, this suggests that arousal enhanced both flexor and extensor activity.

Interestingly, extensor bias has been reported in animal preparations under the influence of locomotor-stimulating neurotransmitters (Jamon et al., 2002). However, Juvin et al. (2007) found that flexor-extensor bursts were symmetrical in fictive locomotion in isolated spinal cord preparations of newborn rats, and proposed that extensor bias in adult rats is caused by sensory feedback provided by limb movement. Perhaps arousal caused a universal change to the newborn locomotive system that made them more sensitive to the peripheral input of the treadmill. It is possible that an increased ability to put weight on the stance leg brought this leg into further extension and increased its extension rate because they could push their body upwards more efficiently.

8.4.8 Are improvements in interlimb coordination and step quality indicative of increased muscle energy?

There are two more results found with high arousal that require explanation. First, interlimb coordination became more alternated with arousal in the first experiment, whereas the movement between the main movement phases of the hip and knee joints
were more synchronous in the second experiment. Are these two effects related, and can they be a function of high arousal? The only suggestion that can be offered is that high arousal decreases lag. Domellöf found that the lag to the first step decreased with arousal (2007). Vecchierini-Blineau and Guihneuc found a lowering of stimulus thresholds and gain in response amplitude for polysynaptic reflexes with crying (1982), although the effects of limb position and muscle contraction levels may have confounded the results. Perhaps, the lag between the two legs decreased in the same way as it did for the muscle bursts. When the muscle is more ready and able to respond, tighter coordination between legs or joints may be afforded, regardless of its mode. This may also have increased parallel stepping in Experiment 2.

The strongest effects were seen for alternation in Experiment 1. Although opposite phasing is more demanding, it is already a behaviour seen in newborns; likely because it offered energetic benefits in the womb. Simultaneous joint action within one leg may have similar intra-uterine benefits. Arousal may have simply increased the expression of two-leg and/or two-joint strategies due to the improved readiness and availability of the muscle.

Secondly, why did step quality improve in Experiment 1? Note that the quality of steps was based on two features: 1) smoother stance phase and/or 2) more forward touchdown, signifying better range of motion during the swing. A smoother stance phase may be a result of improved weight acceptance the leg, particularly on a smooth belt. Increased muscle energy may afford better weight acceptance in itself. But why did swing range of motion increase? Touching the foot down in a further forwards position requires complex muscle timing, which cannot be simply a function of increased muscle readiness and strength. However, if the stance was smoother and longer, forward swing may have been facilitated by the viscoelastic properties of the muscle in the extended position (Thelen, 1986b). Thus, the improved quality of the stance phase could have reinforced the quality of the swing.

8.4.9 Is the relationship between arousal and movement reciprocal?

The literature suggests that movement in itself may increase arousal. This interpretation resulted from the observation of an after-effect in seven-month-old infants who stepped on a moving treadmill (Thelen, 1986b): stepping increased in subsequent trials, even though these did not contain a moving treadmill. Thelen attributed the step increases to a ‘general tonic excitation in response to the previous steps (p. 1504). The current project
did not see an after-effect to stepping on the treadmill. In both experiments, stepping did not improve systematically between the first and the fourth trial, and similarly, mean arousal remained stable. It is possible that the arousal system is not yet very sensitive to movement in newborns. Alternatively, it is possible that the newborns are already primed to a sufficient arousal level by the simple act of picking them up: supporting them under the armpits and stepping may not have had any further arousing influences. This may be different in older infants, who are more habituated to being handled. Therefore, in this experiment, no reciprocal link between stepping and arousal was shown.

8.4.10 The effect of Dycem on the relationship between arousal, stepping and pumping

The idea that arousal affects weight-bearing and thus improves step quality on the treadmill may also clarify why arousal affected movements differently in the first experiment compared to the second, particularly the lack of effect on step rate in Experiment 2. The improvement in pump rate with high arousal in this experiment seems to contrast the findings for stepping on a smooth treadmill (Experiment 1), where steps became further forwards with better stance phases when newborns were more aroused. Did arousal improve the ability to weight-bear, causing the foot to slide less on the smooth treadmill belt, but not on the tacky treadmill belt because the foot could not slide in the first place? This may explain why step rate improved as a function of arousal in the first experiment: it enhanced the effect of the treadmill in a similar way to the tacky surface in the second experiment – by improving foot contact during stance. It may also explain why stepping became ‘better’ in the first experiment but not in the second experiment: arousal did not improve foot contact during stepping in Experiment 2 because it was already optimised.

The relationship between arousal and pump rate on the two different treadmill belts also becomes clearer. Considering that arousal increased movement generally, pump rate should improve alongside step rate. Because pumps are generally characterised by stance phases shorter than half a foot length and have limited belt contact (see coding parameters in Chapter 3 and Appendix A for more detail), improved weight bearing is not likely to further affect pump rate, particularly because in pumps, the initiation of a new movement was not necessarily determined by treadmill induced hip extension. If belt contact had minimal influences in pumping, even with arousal mediated increased weight-bearing, pumping should still increase as a function of arousal in both experiments, whereas
improvements in step rate were not observed in the second experiment because they were already at their maximal rate. Indeed, pump rate increased in both experiments, although it did so more strongly for the first experiment.

8.4.11 The effects of arousal: general improvement of energy but no evidence for increased spinal involvement.

In animal preparations, neurotransmitters and their artificial blockers or agonists have been used to facilitate fictive motion or stepping through the activation of CPGs (Grillner, 1973, Baker et al., 1984, Barriere et al., 2004, Juvin et al., 2007). Because arousal systems facilitate the production of similar neurotransmitters naturally, a shift towards more CPG-like behaviour would not have been strange. However, the evidence did not support this shift, and it is important to remember that arousal-mediated neurotransmitters affect other neural structures as well, and could have assisted an increase in vigour on a broader level.

Stepping is complex and neuromuscular strategies, joint behaviour and end-effector behaviour are affected by many interplaying and mutually inhibiting or reinforcing factors that make every step different. It is unlikely that a spinal pattern generator is the main driver of stepping at any time, aroused or not aroused. However, the expression of certain factors of the step - such as flexion and extension rates, and the tightening of coordinative strategies - may be enabled by arousal by an increase of muscular activation through improved neural transmission. Thus, the current thesis suggests that arousal improves stepping and responsiveness to the treadmill through general improvements of physical readiness for movement.

There is one further aspect to consider in relation to arousal during stepping. When infants are highly aroused, they may step more, but crying also causes multiple unfavourable changes to newborn physiology. Prolonged periods of crying increase stress hormones in the body (Ludington-Hoe et al., 2002) which has been shown to have negative long term effects on the limbic system in rats (Brunson et al., 2001) and possibly also in humans (Anand and Scalzo, 2000, Schore, 1996). Additionally, a negative association with stepping on the treadmill must be avoided, as this may perpetuate as a reluctance to step on the treadmill through infancy. Even though the effect of arousal appears potentially beneficial, arousal should not be allowed to increase to crying levels in infants stepping on the treadmill.
8.5 Accumulated evidence in favour of dynamical systems theory

8.5.1 Pervasive variability

This project confirms that stepping cannot be elicited reliably. However, polysynaptic reflexes can typically be evoked when infants are awake or asleep (Vecchierini-Blineau and Guihneuc, 1982), hence, newborn stepping does not appear to be a reflex. Even when in the most favourable arousal state for reflex excitation, infants do not step reliably. Additionally, stepping does not depend on central pattern generation. CPG theory suggests that stepping is primarily driven by flexible neural networks in the spinal cord and brain stem. CPG and neural network theorists have offered evidence for central patterns in their observations of predictable neuromuscular patterns in stepping (Dominici et al., 2011) and strong linear adaptability to proprioceptive cues (Yang et al., 1998a).

This discussion has highlighted omnipresent variability in muscle responses, joint behaviour, coordination and step rate responses. If stepping was under CPG control, it would have been possible to extract a clear temporal and spatial structure without masking inter and intra-individual variations in the neuromuscular responses through averaging. This would have been reflected in defined peaks and troughs in the POA profiles, or even trough defined flexor-extensor alternation between the swing and stance phase (Dominici et al., 2011). Instead, POA profiles were decidedly flat, and extensor activity even decreased during the stance phase in Experiment 2. Furthermore, the movement of the limbs would have been more stereotypical if muscle output was regulated by CPGs alone: step kinematics would have shown considerably less variability. Lastly, if stepping was primarily driven by neural networks that are sensitive to proprioceptive gating (Pang and Yang, 2000, Yang et al., 1998a), step rates would have shown a more linear and less variable response to treadmill speed. However, step rate decreased for the fast treadmill speed, and step rate responses were so variable that there were no significant differences between speeds. Additionally, the relationship between muscle burst timing and treadmill speed should have been stronger (Yang et al., 1998a). In short, different motor solutions were found for each step separately. Although the treadmill did affect stepping through sensory cues, the existence of CPG function in newborn stepping was not confirmed: sensory adaptation can also occur without the presence of a CPG. The pervasive variability of all responses confirms the influences of multiple contributing factors in newborn stepping.
8.5.2 Optic flow sensitivity

One of these factors was directly shown in this experiment. Even in the presence of a very strong tactile-proprioceptive stimulus, optic flow was still associated to some modifications to step kinematics and neuromuscular behaviour. Although outputs such as step rates were strongly constrained by the mechanical effect of the treadmill, there were changes to joint angles and joint movement coupling when the coherence between optic flow and treadmill was modified. Additionally, a systematic deactivation of muscles was observed at the beginning of the stance phase. Previous research has shown stronger responses to optic flow on static surfaces and in the air (Barbu-Roth et al., 2015, Barbu-Roth et al., 2009, Barbu-Roth et al., 2016, Barbu-Roth et al., 2013), suggesting that the visuo-motor link is subtler than the tactile-proprioceptive link to stepping. This is not strange, as the treadmill effect probably works at a more direct level than the optic flow effect. Even if the effects of optic flow on the treadmill are weak and do not stimulate more advanced behaviour, it does confirm the link between vision and locomotion, and thus the complexity of the control of stepping at birth.

8.6 What is stepping, and can interventions with newborns facilitate an earlier walking onset?

In a natural, non-moving situation, flexion and extension cycles at birth tend to be mainly vertical (Siekerman et al., 2015, Barbu-Roth et al., 2015). Supporting newborns over a moving treadmill made these cycles more step-like, with larger range of motion in the swing and a perceivable stance phase. Forward progression in stepping on a static surface may have been caused by the position in which the infants were supported in this experiment, which was tilted slightly forwards and allowed the swing leg to passively move forward. The kinematic similarities between pumping and other neonatal ‘stepping’ behaviours in different contexts were remarkable. Thelen described the kinematic similarities between supine kicking and upright stepping on a static surface (Thelen and Fisher, 1982). From the descriptions, it appeared that these ‘steps’ had very little forward potential. The fact that newborns make vertical flexion-extension cycles on static surfaces, and do not shift towards forward translation unless the limbs are guided by the treadmill or pulled by gravity while tilted forward, raises the question if the definition ‘stepping’ is not too leading. It is important to view early abilities without attributing them to precocious competency at the future skill (Campos et al., 2008). However, this does not exclude the possibility that these early abilities are functional in a different
context or contribute to the development of a later functional behaviour (Campos et al., 2008). Teulier et al. noted that it was not clear, even in older infants, if stepping reflected ‘a goal of overcoming the instability of having their feet moved backward, beyond their centre of mass’, or a ‘desire to “step” or walk’ (Teulier et al., 2012, p. 858). Perhaps flexion-extension cycles only become ‘steps’ when infants develop intent to move forwards in an upright position.

8.6.1 What is newborn stepping?
The relationship between stepping and later walking is emphasised in the literature. However, it may be more appropriate to look backwards to the period immediately preceding birth. It is likely that stepping at birth is more connected to intra-uterine general movement than it is to adult locomotion. Fortunately, the methods of studying human foetal movement in detail have become more sophisticated, allowing for a better view of the kinematics of movements in the womb, and even brain activity. Combining the information gained from ultrasound studies and more invasive studies with animals might offer some insights into factors that define the features of newborn stepping.

Studies suggest that even from 22 weeks of gestation, human foetuses can make goal directed movements (Zoia et al., 2007, Reissland et al., 2014) and towards the 36th week, movements become more complex and coordinated (Grant-Beuttler et al., 2011). Leg movements are primarily conjugated (parallel) up to week 12-13 of gestation, but alternated leg movements become more prominent after 13 weeks of gestation (Piontelli, 2014), including cycling movements from week 15. Foetal leg movement - or movement in general - occurs to prepare for activity after birth and is essential for the development of muscle and bone. Additionally, movement is required for the development of sensory systems such as the tactile, vestibular and proprioceptive system (Piontelli, 2014). Animal studies have suggested that the coordination between legs is determined by environmental factors; to overcome the elasticity of the womb, extension with one leg at a time or in alternation is more efficient than conjugate extension (Blumberg et al., 2010, Robinson et al., 2006). By and large, the expression of spontaneous foetal leg movements is functional, driven by an intrinsic drive to move and its form is guided by what is most efficient in the uterine environment.

After birth, newborns are introduced to a gravity environment and free space. When supported over a tactile surface, the uterine wall is mimicked by the pressure it exercises onto the feet and legs. It is likely that a static stimulus evokes the most natural stepping
behaviour. In the first experiment of this project, newborns responded to the static tactile stimulus with pumps and single or alternated steps with long step cycles and long stance phases. We propose that stepping in the newborn closely mimics behaviour in the womb, but with modifications and increased variability in response of the introduction of free movement space and gravity (Barbu-Roth et al., 2016, Forma et al., 2015).

In this light, the term ‘stepping’ may not describe the complex nature of these newborn leg movements comprehensively. Although it resembles adult walking, it is important to remember that stepping is a form of infant leg movement that is not spontaneous but constrained to a recognisable form, either by upright support or by a treadmill stimulus. However, because they 1) are functionally linked to the prenatal period, as they are locomotive responses, 2) are continuous throughout infancy and 3) can be utilised to train independent walking, newborn leg movements are not merely random and the term ‘stepping’ is indeed the most suitable.

8.6.2 Is treadmill stepping suitable to train stepping in newborns?

Treadmills, particularly when they are covered with a high-friction material, appear to facilitate stepping in the newborn through a combination of passive mechanical pull to drive the stance phase, and proprioceptive cues from the leg to initiate a swing when it reaches a certain degree of extension. In newborns, the drive to step on the treadmill may be quite dissimilar to the drive to pump on a static surface. In any case, treadmill stepping offers a very different experience to free stepping and it is important to establish how the treadmill offers benefits at birth: do they reinforce adaptive neuromuscular strategies? The answers are not clear. There seems to be no tendency for newborns to adapt their muscle synergies to become more functional for forward walking when stepping on the treadmill stepping or on the optic flow-Dycem enhanced stepping. This may come with experience on the treadmill, as muscle synergies are optimised in repeated perception-action cycles. Exploration in the presence of appropriate sensory information stimulates the formation of adaptive movement behaviour (Teulier et al., 2015). However, does supported stepping on the treadmill give the appropriate environment to discover neuromuscular strategies that are functional for walking? Thelen et al. (1984) suggested that infants lose their ability to step because their legs become unable to support their increasing weight, and Barbu-Roth et al. proposed that particularly the inability to bear weight on one leg suppressed stepping after the first couple of months of life (2015). In this case, extensor participation stepping may be an important function to train in treadmill
stepping. However, it is not clear if, after the initial period of very variable muscle patterns, newborns will start using the extensors in treadmill stepping. Even if they do, it may not be discernible in the ‘messy’ EMG output until much later in infancy (Teulier et al., 2012). Even if extensor participation is not evoked by treadmill stepping, treadmill training at very young ages may have other subtle benefits. Triggering flexion through passive extension may help overcome the inability to lift the legs during the time in which stepping on a static surface can no longer be evoked. Additionally, experiencing the movement of stepping may reinforce perception-action cycles, even if the stance is mostly passive. Starting step training directly after birth may bridge the gap between newborn stepping and more voluntary infant stepping, which may be a crucial period in certain disabilities.

8.6.3 Could optic flow help bring forward independent walking?

In newborns, optic flow stimulation appeared to have an influence on neuromuscular strategy even though the effect on step rate may have been masked. It is not clear if the visuo-locomotor link in newborns resides in the brainstem (Joseph, 2000) or if there is functionality in the visuo-motor cortex, but it certainly spans the spinal cord. This may make step training with optic flow beneficial for infants with a spinal cord injury. But although it was able to modify joint behaviour and the activation of muscles in the presence of a much stronger stimulus, the effects may not have been functional in the support of upright stepping. Firstly, random optic flow was associated with increased pumping behaviour. Although increased pumping behaviour is a positive outcome in newborns, random optic flow has little application in the facilitation of stepping in later life. It does not mimic a moving environment like congruent flow does, which assists in developing the visual system in terms of sensitivity to self-motion. Therefore, non-coherent optic flows, or conflicting conditions, are probably not useful for training paradigms.

Secondly, the deactivation of the extensor during the start of stance seems to be counterproductive, particularly as the extensor seems to be the limiting factor in spontaneous infant stepping. It may be that this only occurs in combination of the treadmill, or Dycem, as there seemed to be no decrease in extensor activity in the experiments of Barbu-Roth and her colleagues (Barbu-Roth et al., 2015, Barbu-Roth et al., 2013). It may be necessary to test stepping with treadmill and optic flow at slower speeds and with and without Dycem to truly distinguish between Dycem and optic flow.
effects - particularly because Dycem and optic flow seem to have similar effects on the neuromuscular organisation in older infants (Pantall et al., 2012). If this is true for newborns as well, optic flow should be the preferred option: congruent optic flow offers a more ecological context for stepping, supports development of the visuo-motor link and helps integrate multiple sensory modalities. However, until more information is available, the current experiments suggest that the use of optic flow on the treadmill seems to be only supportive in infants from about two months of age (Moerchen and Saeed, 2012) and more so when they are older (Pantall et al., 2011, Pantall et al., 2012).

8.6.4 Is it necessary to step on the treadmill from birth?

Now it is clear that newborns respond to treadmill movement with more forward steps, it is important to ask some questions. Firstly, is it necessary for newborns to make forward steps? Perhaps stepping in place under the influence of optic flow is enough to train them. However, perceiving forward steps at an early age may stimulate the emergence of muscle strategies that support stepping forward at a more precocious age – before motivation to go forward is a factor. Additionally, at-risk infants often make few steps if any at all, and treadmill movement may be the only way to increase step rate. Furthermore, the treadmill makes step training much more accessible to parents, involving them more in the intervention process. The treadmill appears to be a beneficial aid in the training of walking.

Secondly, what justifies bringing forward the start of interventions to directly after birth? The plasticity of neuromuscular and corticospinal pathways when humans are young, especially under the influence of activity, offers a ‘window of therapeutic opportunity’ (Eyre, 2007, p. 1146). Current interventions have commenced at the age that infants were able to sit (Ulrich et al., 2001) or when cortical control was presumably established (Schlittler et al., 2011). However, research shows that cortical influences at birth are more advanced than previously thought, especially because corticospinal pathways are present in utero (Eyre et al., 2000), and spontaneous leg movement is influenced by the primary motor cortex even at birth (Kanazawa et al., 2014). As coordinated hand-to-mouth movements accompanied by anticipatory mouth opening was observed in foetuses (Reissland et al., 2014), it is likely that learning is already in progress in the womb (Teulier et al., 2015). Additionally, delaying training for five or six months at minimum reflects missed opportunities at the window of greatest plasticity (Teulier et al., 2015),
particularly for infants with spinal cord injury. The case for very early intervention - as close to birth as possible - seems strong.

Finally, are the sensory stimulations giving the appropriate experiences for newborns with typical development and for each separate at risk group? Depending on the newborn’s pathology, the treadmill training with or without optic flow may be more or less effective (Teulier et al., 2015). Infants with Down syndrome respond well to treadmill training (Ulrich et al., 2001; Wu et al., 2007; Ulrich et al., 2008; Wu et al., 2010), but there is limited evidence with infants with cerebral palsy or spina bifida. This may partially be due to a lack of longitudinal training studies (Teulier et al., 2013), but intrinsic difficulties may also lie within the way the defect interferes on the development of stepping into walking. In spina bifida, there is a neural tube defect, often complicated by hydrocephalus or other comorbidities. In cerebral palsy, there may be problems in supraspinal structures. If treadmill training is to positively affect the neural transmission and efficiency and flexibility of the developing pathways, it must specifically target the problem area. For example, the stimulating effect from optic flow may be less accessible to infants with a compromised spinal cord, and yet, it is probably quite important to strengthen this link aggressively. Overall, it is not clear how much the treadmill reinforces cortico-spinal pathways in stepping. It is possible that additional sensory stimuli play an important role here. More research is needed to clarify the effects.

8.7 Limitations

The main limitation with the current project is the high attrition rate, leading to low sample sizes in certain analyses. Drop-out occurred throughout all aspects of the experiment. This was not limited to having to stop the testing because newborns fell asleep or were over-aroused: primary attrition accounted for 25% in the first experiment and 17% in the second experiment. The main problem was caused by the fact that four minutes of testing did not give enough data to compute results for 1) both stepping and pumping at 2) all four different conditions while 3) displaying both crying and non-crying behaviour. This reduced the sample size of some variables (n = 12 in the coordination analysis and n = 9 for the step cycle analysis in Experiment 1). Additionally, there were methodological problems that were not anticipated. For example, it was necessary to eliminate a fully complying subject from the analysis because an ankle marker did not track properly. Furthermore, if one electrode was lost during one trial, the entire subject could not be used.
Low sample size increased the likelihood that significant differences may have been overlooked in some of the sub-analyses. Post hoc power of the main results (step and pump rates at different treadmill speeds and optic flow conditions) calculated to 0.75 for Experiment 1 and 0.60 for Experiment 2. It is recommended that the reader interprets the results while keeping in mind that type II error may have occurred in the sub-analyses across the two studies, and that type I error may be present in the kinematics and the EMG analyses, particularly if an isolated effect is observed. Although all significant results were presented in the text of the results sections, the discussion focused mainly on trends within the data, whilst acknowledging the role of chance in stand-alone findings.

Perhaps the project was too ambitious. However, the main outcomes for treadmill speed and optic flow are powerful enough to show differences at reasonable confidence, and the exploratory data fill knowledge gaps and show trends that may be further explored in more specific experiments. Additionally, the fact that there was considerable primary drop-out does not suggest that newborns in general do not respond well to stepping on the treadmill or with optic flow. Attrition due to arousal state is quite normal in newborn research and in a training paradigm, the effects can be minimised by trying again at a different time of the day, when the infant in a more appropriate behavioural state. In future experiment, it is recommended to recruit at least twice the current sample size, approximately 40 babies, and up to 72 when analysing EMG in detail. Fewer variables can be tested: it is now clear in which areas differences may be expected.

Although the Dycem effect was a result in itself, it also masked some of the effects the optic flow might have had on stepping behaviour. Additionally, it confused the 3D motion capture system considerably and caused poor marker tracking. An experiment on optic flow and the treadmill is needed to investigate the effect of visual stimulation together with a weaker tactile/proprioceptive stimulus, as the effects of Dycem and optic flow could not be parsed completely. The additional effect of changing the treadmill surface to a smooth white belt would also reduce the reflection of the infrared light in the 3D data collection, improving the tracking of the markers.

From the analysis of the kinematics and EMG, many things can be learned. Firstly, there was a considerable amount of noise in the EMG signal some newborns. Typically, the signal-to-noise ratio is low in infants. It is essential to ensure low skin impedance before testing babies, and a base-line trial to verify whether clear EMG, including reciprocal activation, can be captured is strongly recommended. Secondly, the electrode cable fixations obstructed the view of the markers in the second experiment. Tubular netting
was used to reduce wire sway. Telemetry system to collect EMG data may eliminate the need to tie up cables. If not available, possible electrode fixations must be above the knee to avoid masking of the ankle marker.

Further, the absence of kinematic measures in the first experiment was unfortunate. It would have been interesting to contrast the effect of treadmill movement and arousal on extension in a less strongly defining environment without Dycem. Future studies should ensure that both EMG and 3D kinematics are available for a detailed analysis and to contrast the findings with results from frame-by-frame video coding. It is clear that sensory stimulation affects stepping on many different levels; the interplay between muscle behaviour and end-effector behaviour gives important insights into the organisation and control of movement behaviour.

As a last point, it should be noted that although it was clear that variability was very high throughout both experiments, no quantifying measures of variability were reported in this thesis, excepting the POA in the EMG analysis. True measures of variability would have been able to give more information on the characteristics of neonatal stepping.

8.8 Future research

From the results found in the two studies, new questions arose. Firstly, the use of Dycem had a clear effect on stepping, but the details of this effect should be further studied in a design with stepping on a Dycem enhanced treadmill contrasting stepping on a treadmill without Dycem. More importantly, the change of the Dycem effect on neuromuscular behaviour across age groups may give an indication from which Dycem will give experiences beneficial to the development of forward stepping.

More importantly, excluding Dycem from a study on stepping on the treadmill with and without congruent optic flow on a moving and a static treadmill may target the question in this thesis better than the current experimental design. It is still not clear what effect optic flow would have had in a less defining environment. A smooth treadmill belt would likely allow more modification from the optic flow on a superficial level. Additionally, slower treadmill speeds may have the same effect, because they are closer to the natural environment than a fast-moving treadmill. It would also be interesting to see if optic flow may cause changes to the currently examined parameters on pumping and stepping separately.

Lastly, it is not clear if bringing forward the start date of interventions has actual benefits on infants with TD or at-risk groups. The results of this project suggest that training
stepping with a smooth treadmill and optic flow does not reinforce a reflex or constrain neuromuscular behaviour towards rigid movement patterns. Therefore, a careful longitudinal study with infants with TD, and infants at risk of developmental delay might close that knowledge gap. It will be particularly interesting to see if starting training with optic flow might reinforce the visuo-motor link in infants with a spinal cord lesion, and if training the visuo-motor link in a congruent context from a young age enhances the development of balance in locomotion in the general population.

8.9 Conclusion
The current project found that newborns responded to treadmill movement with more forward stepping movements, although they did not adapt to treadmill speed as strongly as their older peers would. Differences in cycle duration, stance ratio and muscle burst length occurred between the moving treadmill conditions and the static condition alone, and further neuromuscular effects were variable. Optic flow on the treadmill did not affect step rate, quality or coordination, but effects were found at the kinematic and neuromuscular level. Congruent optic flow was associated with less synchrony in joint movement and less hip extension and faster optic flow deactivated the majority of the muscles during stance. Arousal stimulated leg movements: on the smooth treadmill, all movements increased when infants were crying, and step quality and coordination was more advanced. On the treadmill with optic flow, high arousal was associated with an increase in pumps, improved step extension and increased inter-joint coupling. The EMG analysis did not show more effective neuromuscular strategies as a result of arousal in either experiment although there was a general increased likelihood of activation for proximal muscles. Overall, the responses were highly variable for step rate, kinematics and muscle behaviour alike.

The link between vision and stepping on the treadmill at birth, and the need to offer a rich sensory environment in rehabilitation, justify the introduction of the treadmill and optic flow at birth. When utilising optic flow on the treadmill, it may be wise to use slow speeds on a smooth belt initially. Arousal can help to increase the volume of stepping experience by generic stimulation of the most proximal muscles in particular, promoting weight bearing and increasing the treadmill effect. It is still not entirely clear if arousal restricts the variability of the behaviour. However, a negative association with the intervention must be avoided, suggesting that newborns should not be so aroused that they are crying while stepping.
There are still areas that need to be uncovered. Firstly, tactile-proprioceptive and visual sensitivity may be more easily revealed in newborns if the stimuli are less strong and ideally, the experiment would be repeated using slower treadmill speeds and optic flows. Additionally, the effects of Dycem in newborn treadmill stepping need to be uncovered in a separate experiment. Further, the effects of treadmill training from birth have not been recorded in a longitudinal study, therefore the long-term effects are not confirmed. It is not entirely clear if training paradigms using the treadmill from birth with or without optic flow have benefits for certain at-risk groups, and how the mechanism of training interacts with the limitations caused by the disability. However, this project has shown that it is possible to facilitate stepping in newborns utilising the treadmill and optic flow, which may open up multiple possible directions for treadmill use in at-risk populations in the future.
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Appendix A: Coding guidelines

The first section (start movement) looks at general movement specifics

1.1 First movement time
- Fill out the first time the toe moves
- Off the belt
- For more than 0.5 s
- Obviously against gravity
- For ‘shudder’ steps: code the first time the toe moves against the belt

1.2 Step laterality
- Fill in if the movement was on the left or on the right. Code if with both legs at the same time (parallel):
  - If the second leg follows the first leg (approximately), within 20% of the previous movement cycle, code as ‘both’
  - Code the start movement for the lead leg, i.e., the leg that makes the most obvious movement
  - Incorporate all movements such as small glides and pumps into the parallel step if it appears roughly in sequence with the motion
  - The contralateral foot has to touch the belt or move in conjunction with ipsilateral leg
  - Be careful, if movement of the contralateral leg finishes after the lead leg, allow for that movement to finish before coding a new movement on that side

1.5 Step type
- Note which type of foot movement was made
- Note: in case of a parallel movement, code the most obvious movement

1.5.1 Step:
- It looks like a step in real-time
- Most distal point of the toe moves from behind the mid-line (determined by hip marker) to in front of (or in line with) the mid-line
- There has to be a stance phase (stance longer than one foot length)
- In case of a borderline step: if both step distance and stance time are borderline, it should not be coded as a step, but if only one parameter is violated, tick ATYPICAL.
- A step with flexion times over 3 seconds is a pump
- Foot does not have to leave the belt: swing can be gliding

1.5.2 Pump
- Foot clearly lifts off the belt
- Foot does not pass through the mid-line: it is a straight ‘up-down’ motion
- Step with flexion exceeding 3 seconds
- Code end movement time when foot reverses downward motion

The next section (stance) looks at the stance phase-ONLY CODE FOR STEPS

2.1 Touch-down time
Note the first frame that the foot makes contact with the belt again
Code the frame before the foot starts moving back

2.2 Touch-down position
- Note the site of first contact with the belt.
- TOE: toe touches down first very obviously
- HEEL: heel touches down first very obviously
- FLAT: mid-foot touchdown with the heel touching the belt

The following section (end movement) codes the end of the movement

3 End movement time
- Note the first frame in which the foot stops moving with the belt
- The foot may be lifted off the belt
- The foot can start sliding over the belt but remain in the same position
- Make sure there are no missing frames in the alternating stepping sequence: end movement time is the frame just before the next step commences
- For a parallel sequence: the first leg that stops moving backwards should be coded as end movement time

The last section (sequence) looks at alternation-ONLY CODE FOR STEPS

4 Step Sequence
Note step sequence

4.1 Single Step
- Step was a single occurrence
- Repeated steps on the same side

4.2 Parallel step
- Step overlapped step on the contra-lateral side
- The legs move in a paired fashion
- Both legs followed approximately the same movement, but one leg may have shown less obvious step characteristics

4.3 Alternated step
- Step was closely preceded or followed by a step of the contra-lateral side (greater than 20% but less than 80% interlimb phase lag).
- One foot must show stance when the other is airborne
- Verify step by step with the alternation tool
- Only steps are allowed to alternate
- If a step is alternate and includes a double step, code the leg that shows the double step as ‘double’. However, the other step can alternate with it.
- If in doubt, look at the functionality of the sequence: was the infant able to control the legs separately?

4.4 Serial step
- Step alternated with a step on the contralateral side, but had a phase lag larger than 80%

215
• Contralateral step had to start within 1 second of the end of the ipsilateral step

4.5 Shuffle
• Stance phase of a step shuddered (i.e., stopped briefly, lifted off the belt for less than 0.5 seconds)
• Alternatively, the step had not passed through the mid-line yet but moved back into a new stance phase
• DO NOT CODE STANCE
• Tick ATYPICAL

Borderline decisions
• If a decision is borderline, follow the procedure:
  • Mark and verify it after a period of time
  • If the coding is not the same, mark again and verify again after a period of time
  • If 2 decisions were the same, choose this option, if not, mark for coding by a second coder
  • If second coder is unsure and no agreement can be found, do not code the event.
  • It is important to look at the functional movement and the grander scheme of things if movements are up for discussion…

*If a swing phase is completed but stance it not completed before the end of the trial, count it a step but note only the frame numbers for events occurring within the trial duration

**Be careful not to code stepping behaviours that occur during periods when the experimenter is repositioning the infant. During the first few coding sessions it may be easier to view the trial first to determine repositioning periods and to list them as a check for yourself

***If step occurs before trial begins, it does not count. If step begins before end of trial it counts as a step but don’t include any events (e.g. touchdown, end of stance) that occur after trial ends.
Figure 4.1: Coding flow chart

Does the foot move?
- On the floor, or
- For more than 0.5 s, or
- Against gravity

Code first movement time
Code abnormality
Code trunk and hip angle

Do(s) it look like a step in real time?

If foot or body also go straight to PUMP:

Yes
- Did the foot cross the midline?
- Was stance longer than one full length?

Yes to both:
- CODE TD time and post
- CODE movement: STEP

Yes to ONLY ONE:
- CODE TD time
- CODE movement: STEP
- Add ATYPICAL

No
- Did the foot go up and touch down in the same place?

Yes:
- CODE movement: PUMP

CODE end movement time
For pump: upon first touchdown
For other: when movement stops, or when following separate movement starts (normally after movement has gone forwards, then backwards)
For step: when foot stops moving backwards on the frame before a new step-off (in a sequence)
Appendix B: A breakdown of excluded data

The following tables describe the total seconds of data utilised for the final analysis, per analysis and per experiment (Figures 14 to 17). Each vertical subsection describes, per condition, 1) trial length (excluding time used to replace infants and when a parent was touching the infant) and 2) whether the infant provided data for a certain analysis (movement type analysis, quality analysis, coordination analysis and cycle analysis) during that condition, which is recorded with a tick mark when positive. Only when every condition contributed data for a given variable, the subject was included for the analysis of this variable. For example, in subject 2, the boxes for all trials were ticked for the movement type analysis: the subject was included in this analysis. However, for the quality analysis, the static trial did not contribute data because the subject made no steps in this condition. Therefore, the entire subject was excluded for this variable. The rightmost column shows overall testing time used for analysis per subject, and in Tables 15 and 17, the amount of time spent in state 1, 2 and 3 is recorded. The tables also supply information on the exclusion of the entire subject due to under or over-arousal. Additionally, the time of perceivable eye-opening is described in Figure 18, and in Figure 19, included kinematic data from steps and pumps was expressed as a percentage of total movement number. Figure 20 lists the number of steps utilised to calculate POA across static and dynamic trials.
Table 14: Overview of analysed time and available data for the movement-by-condition analysis in Experiment 1

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Table 15: Overview of analysed time and available data for the movement-by-arousal analysis in Experiment 1

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Table 18: Overview of total perceivable eye-opening time in Experiment 2

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Note: It was difficult obtaining a measure of true time of eye-opening because newborns tend to open their eyes a very small amount (i.e., squint) when they are relaxed. It is therefore not clear to what extent each newborn was exposed to the optic flow.
Table 19: Included data for the kinematics analysis: analysed movements as a percentage of total movements

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Table 20: Steps contributing to the POA figure between static and dynamic conditions

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Appendix C: Individual profiles of the kinematic variables

Figure 42: The effects of optic flow condition and arousal on step and pump length
Figure 43: The effects of optic flow condition on hip angles
Figure 44: The effects of optic flow condition on knee angles
Figure 45: The effects of arousal level on hip angles
Figure 46: The effects of arousal level on knee angles
Figure 47: The effects of optic flow condition and arousal levels on hip and knee range of motion
Figure 48: The effects of optic flow condition on angular velocity of the hip
Figure 49: The effects of optic flow condition on angular velocity of the knee
Figure 50: The effects of arousal level on angular velocities of the hip
Figure 51: The effects of arousal level on angular velocities of the knee
Figure 52: The effects of optic flow condition on joint synchronicity
Figure 53: The effects of arousal level on joint synchronicity
Figure 54: The effects of optic flow condition and arousal level on movement units in steps and pumps
Appendix D: Inferential statistics from the probability of activation (POA) analysis

Table 21: Statistics for POA of the all muscles in Experiment 1, for swing and stance phase for static and dynamic conditions. Note that there are five time windows (bins) for the swing phase (from 0-100% of the swing phase in 20% increments) and 10 time windows for the stance phase (0-100% of the stance phase in 10% increments).

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<th>N</th>
<th>P-value</th>
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<td>-0.051</td>
<td>11</td>
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</tr>
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<td>-1.245</td>
<td>11</td>
<td>0.213</td>
<td>0.100</td>
</tr>
<tr>
<td>Bin 4 (60-80%)</td>
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</tr>
<tr>
<td>Bin 1 (0-10%)</td>
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<td></td>
<td>-1.423</td>
<td>11</td>
<td>0.155</td>
<td>0.500</td>
</tr>
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<td>Bin 2 (10-20%)</td>
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<td>-0.311</td>
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<td>-0.445</td>
<td>11</td>
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<td>0.352</td>
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<td>-1.070</td>
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<td>-0.578</td>
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<td>0.563</td>
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<td>Bin 7 (60-70%)</td>
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<td></td>
<td>-0.934</td>
<td>11</td>
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<tr>
<td>Bin 8 (70-80%)</td>
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<td>0.450</td>
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<td>-0.623</td>
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<td>Bin 1 (0-20%)</td>
<td>Bin 2 (20-40%)</td>
<td>Bin 3 (40-60%)</td>
<td>Bin 4 (60-80%)</td>
<td>Bin 5 (80-100%)</td>
<td>Bin 1 (0-10%)</td>
</tr>
<tr>
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<td>0.878</td>
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<td>0.350</td>
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<td><strong>Stance</strong></td>
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<tr>
<td><strong>Swing</strong></td>
<td>-1.379</td>
<td>0.168</td>
<td>0.833</td>
<td>0.551</td>
<td>-2.293</td>
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<td><strong>Stance</strong></td>
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<td>-0.663</td>
<td>0.508</td>
</tr>
</tbody>
</table>
Table 22: Statistics for POA of the Biceps Femoris in Experiment 1, for swing and stance phase and by condition and arousal state. Note that there are five time windows (bins) for the swing phase (from 0-100% of the swing phase in 20% increments) and 10 time windows for the stance phase (0-100% of the stance phase in 10% increments).

<table>
<thead>
<tr>
<th>Bin</th>
<th>χ²</th>
<th>N</th>
<th>P-value</th>
<th>Medians</th>
<th>Z-value</th>
<th>χ²</th>
<th>N</th>
<th>P-value</th>
<th>Z-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Static</td>
<td>Slow</td>
<td>Medium</td>
<td>Fast</td>
<td></td>
<td>Crying</td>
</tr>
<tr>
<td>Swing</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bin 1 (0-20%)</td>
<td>2.169</td>
<td>8</td>
<td>0.538</td>
<td>0.733</td>
<td>0.700</td>
<td>0.435</td>
<td>0.564</td>
<td>-1.726</td>
<td>13</td>
</tr>
<tr>
<td>Bin 2 (20-40%)</td>
<td>1.732</td>
<td>8</td>
<td>0.630</td>
<td>0.733</td>
<td>0.667</td>
<td>0.491</td>
<td>0.548</td>
<td>-1.852</td>
<td>13</td>
</tr>
<tr>
<td>Bin 3 (40-60%)</td>
<td>1.500</td>
<td>8</td>
<td>0.682</td>
<td>0.432</td>
<td>0.367</td>
<td>0.354</td>
<td>0.464</td>
<td>-0.801</td>
<td>13</td>
</tr>
<tr>
<td>Bin 4 (60-80%)</td>
<td>2.115</td>
<td>8</td>
<td>0.549</td>
<td>0.527</td>
<td>0.333</td>
<td>0.388</td>
<td>0.528</td>
<td>-2.344</td>
<td>13</td>
</tr>
<tr>
<td>Bin 5 (80-100%)</td>
<td>6.423</td>
<td>8</td>
<td>0.093</td>
<td>0.450</td>
<td>0.333</td>
<td>0.528</td>
<td>0.667</td>
<td>-1.642</td>
<td>13</td>
</tr>
<tr>
<td>Stance</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bin 1 (0-10%)</td>
<td>6.273</td>
<td>8</td>
<td>0.099</td>
<td>0.325</td>
<td>0.217</td>
<td>0.546</td>
<td>0.708</td>
<td>-1.642</td>
<td>13</td>
</tr>
<tr>
<td>Bin 2 (10-20%)</td>
<td>6.273</td>
<td>8</td>
<td>0.099</td>
<td>0.382</td>
<td>0.217</td>
<td>0.464</td>
<td>0.583</td>
<td>-1.852</td>
<td>13</td>
</tr>
<tr>
<td>Bin 3 (20-30%)</td>
<td>8.065</td>
<td>8</td>
<td>0.099</td>
<td>0.382</td>
<td>0.083</td>
<td>0.464</td>
<td>0.528</td>
<td>-0.874</td>
<td>13</td>
</tr>
<tr>
<td>Bin 4 (30-40%)</td>
<td>4.216</td>
<td>8</td>
<td>0.239</td>
<td>0.325</td>
<td>0.265</td>
<td>0.464</td>
<td>0.472</td>
<td>-0.874</td>
<td>13</td>
</tr>
<tr>
<td>Bin 5 (40-50%)</td>
<td>2.676</td>
<td>8</td>
<td>0.444</td>
<td>0.325</td>
<td>0.230</td>
<td>0.464</td>
<td>0.417</td>
<td>-1.098</td>
<td>13</td>
</tr>
<tr>
<td>Bin 6 (50-60%)</td>
<td>6.452</td>
<td>8</td>
<td>0.092</td>
<td>0.382</td>
<td>0.230</td>
<td>0.500</td>
<td>0.375</td>
<td>-1.255</td>
<td>13</td>
</tr>
<tr>
<td>Bin 7 (60-70%)</td>
<td>2.910</td>
<td>8</td>
<td>0.406</td>
<td>0.183</td>
<td>0.272</td>
<td>0.500</td>
<td>0.431</td>
<td>-1.255</td>
<td>13</td>
</tr>
<tr>
<td>Bin 8 (70-80%)</td>
<td>7.456</td>
<td>8</td>
<td>0.059</td>
<td>0.450</td>
<td>0.212</td>
<td>0.500</td>
<td>0.333</td>
<td>-1.423</td>
<td>13</td>
</tr>
<tr>
<td>Bin 9 (80-90%)</td>
<td>8.719</td>
<td>8</td>
<td>0.033</td>
<td>0.550</td>
<td>0.150</td>
<td>0.393</td>
<td>0.236</td>
<td>-1.412</td>
<td>13</td>
</tr>
<tr>
<td>Bin 10 (90-100%)</td>
<td>1.375</td>
<td>8</td>
<td>0.711</td>
<td>0.450</td>
<td>0.333</td>
<td>0.417</td>
<td>0.261</td>
<td>-1.177</td>
<td>13</td>
</tr>
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</table>
Table 23: Statistics for the POA of the Lateral Gastrocnemius in Experiment 1, for swing and stance phase and by condition and arousal state. Note that there are five time windows (bins) for the swing phase (from 0-100% of the swing phase in 20% increments) and 10 time windows for the stance phase (0-100% of the stance phase in 10% increments).

<table>
<thead>
<tr>
<th>By Condition</th>
<th>By Arousal State</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>χ²</strong> N</td>
<td><strong>P-value</strong></td>
</tr>
<tr>
<td><strong>Swing</strong></td>
<td><strong>Static</strong></td>
</tr>
<tr>
<td>Bin 1 (0-20%)</td>
<td>2.045 8</td>
</tr>
<tr>
<td>Bin 2 (20-40%)</td>
<td>3.792 8</td>
</tr>
<tr>
<td>Bin 3 (40-60%)</td>
<td>1.960 8</td>
</tr>
<tr>
<td>Bin 4 (60-80%)</td>
<td>3.792 8</td>
</tr>
<tr>
<td>Bin 5 (80-100%)</td>
<td>6.592 8</td>
</tr>
<tr>
<td><strong>Stance</strong></td>
<td><strong>Static</strong></td>
</tr>
<tr>
<td>Bin 1 (0-10%)</td>
<td>4.169 8</td>
</tr>
<tr>
<td>Bin 2 (10-20%)</td>
<td>3.118 8</td>
</tr>
<tr>
<td>Bin 3 (20-30%)</td>
<td>4.519 8</td>
</tr>
<tr>
<td>Bin 4 (30-40%)</td>
<td>0.494 8</td>
</tr>
<tr>
<td>Bin 5 (40-50%)</td>
<td>4.831 8</td>
</tr>
<tr>
<td>Bin 6 (50-60%)</td>
<td>3.122 8</td>
</tr>
<tr>
<td>Bin 7 (60-70%)</td>
<td>3.273 8</td>
</tr>
<tr>
<td>Bin 8 (70-80%)</td>
<td>2.805 8</td>
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<tr>
<td>Bin 9 (80-90%)</td>
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<tr>
<td>Bin 10 (90-100%)</td>
<td>1.105 8</td>
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Table 24: Statistics for the POA of the Rectus Femoris in Experiment 1, for swing and stance phase and by condition and arousal state. Note that there are five time windows (bins) for the swing phase (from 0-100% of the swing phase in 20% increments) and 10 time windows for the stance phase (0-100% of the stance phase in 10% increments).

<table>
<thead>
<tr>
<th>Bin</th>
<th>Swing (%)</th>
<th>( \chi^2 )</th>
<th>N</th>
<th>P-value</th>
<th>Medians</th>
<th>Z-value</th>
<th>N</th>
<th>P-value</th>
<th>Medians</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>Slow</td>
<td>Medium</td>
<td>Fast</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bin 1 (0-20%)</td>
<td>2.143</td>
<td>7</td>
<td>0.543</td>
<td>0.455</td>
<td>0.667</td>
<td>0.500</td>
<td>12</td>
<td>0.021</td>
<td>0.673</td>
</tr>
<tr>
<td>Bin 2 (20-40%)</td>
<td>1.164</td>
<td>7</td>
<td>0.762</td>
<td>0.500</td>
<td>0.500</td>
<td>0.429</td>
<td>12</td>
<td>0.050</td>
<td>0.539</td>
</tr>
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<td>Bin 3 (40-60%)</td>
<td>0.500</td>
<td>7</td>
<td>0.919</td>
<td>0.500</td>
<td>0.368</td>
<td>0.444</td>
<td>12</td>
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<td>0.477</td>
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<tr>
<td>Bin 4 (60-80%)</td>
<td>1.136</td>
<td>7</td>
<td>0.768</td>
<td>0.455</td>
<td>0.333</td>
<td>0.296</td>
<td>12</td>
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<td>0.380</td>
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<td>Bin 5 (80-100%)</td>
<td>0.582</td>
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<td>0.901</td>
<td>0.500</td>
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<td>0.556</td>
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<td>0.562</td>
</tr>
<tr>
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<td></td>
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<td>Slow</td>
<td>Medium</td>
<td>Fast</td>
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<tr>
<td>Bin 1 (0-10%)</td>
<td>0.682</td>
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<td>0.877</td>
<td>0.500</td>
<td>0.400</td>
<td>0.556</td>
<td>12</td>
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<td>0.606</td>
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<td>1.095</td>
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<td>0.778</td>
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<td>0.500</td>
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<td>0.500</td>
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<td>0.023</td>
<td>0.652</td>
</tr>
<tr>
<td>Bin 4 (30-40%)</td>
<td>1.836</td>
<td>7</td>
<td>0.607</td>
<td>0.455</td>
<td>0.333</td>
<td>0.500</td>
<td>12</td>
<td>0.034</td>
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<td>Bin 5 (40-50%)</td>
<td>4.762</td>
<td>7</td>
<td>0.190</td>
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<td>0.421</td>
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<td>12</td>
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<tr>
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<td>4.500</td>
<td>7</td>
<td>0.212</td>
<td>0.250</td>
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<td>0.500</td>
<td>12</td>
<td>0.019</td>
<td>0.785</td>
</tr>
<tr>
<td>Bin 7 (60-70%)</td>
<td>5.741</td>
<td>7</td>
<td>0.125</td>
<td>0.250</td>
<td>0.474</td>
<td>0.600</td>
<td>12</td>
<td>0.050</td>
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<td>4.603</td>
<td>7</td>
<td>0.203</td>
<td>0.273</td>
<td>0.474</td>
<td>0.600</td>
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<td>2.077</td>
<td>7</td>
<td>0.557</td>
<td>0.500</td>
<td>0.333</td>
<td>0.667</td>
<td>12</td>
<td>0.038</td>
<td>0.696</td>
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<tr>
<td>Bin 10 (90-100%)</td>
<td>0.785</td>
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<td>0.853</td>
<td>0.500</td>
<td>0.600</td>
<td>0.667</td>
<td>12</td>
<td>0.182</td>
<td>0.741</td>
</tr>
</tbody>
</table>
Table 25: Statistics for the POA of the Tibialis Anterior in Experiment 1, for swing and stance phase and by condition and arousal state. Note that there are five time windows (bins) for the swing phase (from 0-100% of the swing phase in 20% increments) and 10 time windows for the stance phase (0-100% of the stance phase in 10% increments).

<table>
<thead>
<tr>
<th>By Condition</th>
<th>By Arousal State</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \chi^2 )</td>
</tr>
<tr>
<td><strong>Swing</strong></td>
<td></td>
</tr>
<tr>
<td>Bin 1 (0-20%)</td>
<td>9.269</td>
</tr>
<tr>
<td>Bin 2 (20-40%)</td>
<td>3.333</td>
</tr>
<tr>
<td>Bin 3 (40-60%)</td>
<td>3.962</td>
</tr>
<tr>
<td>Bin 4 (60-80%)</td>
<td>2.792</td>
</tr>
<tr>
<td>Bin 5 (80-100%)</td>
<td>6.731</td>
</tr>
<tr>
<td><strong>Stance</strong></td>
<td></td>
</tr>
<tr>
<td>Bin 1 (0-10%)</td>
<td>7.519</td>
</tr>
<tr>
<td>Bin 2 (10-20%)</td>
<td>0.318</td>
</tr>
<tr>
<td>Bin 3 (20-30%)</td>
<td>0.584</td>
</tr>
<tr>
<td>Bin 4 (30-40%)</td>
<td>2.393</td>
</tr>
<tr>
<td>Bin 5 (40-50%)</td>
<td>1.958</td>
</tr>
<tr>
<td>Bin 6 (50-60%)</td>
<td>2.451</td>
</tr>
<tr>
<td>Bin 7 (60-70%)</td>
<td>6.441</td>
</tr>
<tr>
<td>Bin 8 (70-80%)</td>
<td>2.440</td>
</tr>
<tr>
<td>Bin 9 (80-90%)</td>
<td>1.800</td>
</tr>
<tr>
<td>Bin 10 (90-100%)</td>
<td>3.113</td>
</tr>
</tbody>
</table>
Table 26: Statistics for the POA of the Biceps Femoris in Experiment 2, for swing and stance phase and by condition and arousal state. Note that there are five time windows (bins) for the swing phase (from 0-100% of the swing phase in 20% increments) and 10 time windows for the stance phase (0-100% of the stance phase in 10% increments).

<table>
<thead>
<tr>
<th></th>
<th>By Condition</th>
<th>By Arousal State</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>N</td>
</tr>
<tr>
<td><strong>Swing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bin 1 (0-20%)</td>
<td>0.227</td>
<td>18</td>
</tr>
<tr>
<td>Bin 2 (20-40%)</td>
<td>0.397</td>
<td>18</td>
</tr>
<tr>
<td>Bin 3 (40-60%)</td>
<td>2.041</td>
<td>18</td>
</tr>
<tr>
<td>Bin 4 (60-80%)</td>
<td>1.142</td>
<td>18</td>
</tr>
<tr>
<td>Bin 5 (80-100%)</td>
<td>1.414</td>
<td>18</td>
</tr>
<tr>
<td><strong>Stance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bin 1 (0-10%)</td>
<td>8.089</td>
<td>18</td>
</tr>
<tr>
<td>Bin 2 (10-20%)</td>
<td>8.125</td>
<td>18</td>
</tr>
<tr>
<td>Bin 3 (20-30%)</td>
<td>3.898</td>
<td>18</td>
</tr>
<tr>
<td>Bin 4 (30-40%)</td>
<td>1.280</td>
<td>18</td>
</tr>
<tr>
<td>Bin 5 (40-50%)</td>
<td>1.446</td>
<td>18</td>
</tr>
<tr>
<td>Bin 6 (50-60%)</td>
<td>0.772</td>
<td>18</td>
</tr>
<tr>
<td>Bin 7 (60-70%)</td>
<td>1.347</td>
<td>18</td>
</tr>
<tr>
<td>Bin 8 (70-80%)</td>
<td>1.765</td>
<td>18</td>
</tr>
<tr>
<td>Bin 9 (80-90%)</td>
<td>0.754</td>
<td>18</td>
</tr>
<tr>
<td>Bin 10 (90-100%)</td>
<td>2.686</td>
<td>18</td>
</tr>
</tbody>
</table>
Table 27: Statistics for the POA of the Lateral Gastrocnemius in Experiment 2, for swing and stance phase and by condition and arousal state. Note that there are five time windows (bins) for the swing phase (from 0-100% of the swing phase in 20% increments) and 10 time windows for the stance phase (0-100% of the stance phase in 10% increments).

<table>
<thead>
<tr>
<th>By Condition</th>
<th>By Arousal State</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>χ²</strong></td>
<td><strong>N</strong></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Bin 1 (0-20%)</td>
<td>2.890</td>
</tr>
<tr>
<td>Bin 2 (20-40%)</td>
<td>1.845</td>
</tr>
<tr>
<td>Bin 3 (40-60%)</td>
<td>2.547</td>
</tr>
<tr>
<td>Bin 4 (60-80%)</td>
<td>1.264</td>
</tr>
<tr>
<td>Bin 5 (80-100%)</td>
<td>2.702</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Swing</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Lateral Gastrocnemius</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bin 1 (0-10%)</td>
<td>3.184</td>
<td>17</td>
<td>0.364</td>
<td>0.375</td>
<td>0.333</td>
<td>0.357</td>
<td>0.333</td>
<td>-0.524</td>
<td>13</td>
</tr>
<tr>
<td>Bin 2 (10-20%)</td>
<td>3.868</td>
<td>17</td>
<td>0.276</td>
<td>0.364</td>
<td>0.273</td>
<td>0.333</td>
<td>0.167</td>
<td>-1.177</td>
<td>13</td>
</tr>
<tr>
<td>Bin 3 (20-30%)</td>
<td>4.547</td>
<td>17</td>
<td>0.208</td>
<td>0.273</td>
<td>0.273</td>
<td>0.214</td>
<td>0.063</td>
<td>-0.784</td>
<td>13</td>
</tr>
<tr>
<td>Bin 4 (30-40%)</td>
<td>3.247</td>
<td>17</td>
<td>0.355</td>
<td>0.273</td>
<td>0.250</td>
<td>0.143</td>
<td>0.063</td>
<td>-0.035</td>
<td>13</td>
</tr>
<tr>
<td>Bin 5 (40-50%)</td>
<td>1.882</td>
<td>17</td>
<td>0.597</td>
<td>0.333</td>
<td>0.250</td>
<td>0.200</td>
<td>0.143</td>
<td>-1.098</td>
<td>13</td>
</tr>
<tr>
<td>Bin 6 (50-60%)</td>
<td>1.181</td>
<td>17</td>
<td>0.758</td>
<td>0.273</td>
<td>0.182</td>
<td>0.286</td>
<td>0.250</td>
<td>-1.177</td>
<td>13</td>
</tr>
<tr>
<td>Bin 7 (60-70%)</td>
<td>0.244</td>
<td>17</td>
<td>0.970</td>
<td>0.273</td>
<td>0.182</td>
<td>0.286</td>
<td>0.250</td>
<td>-1.983</td>
<td>13</td>
</tr>
<tr>
<td>Bin 8 (70-80%)</td>
<td>0.306</td>
<td>17</td>
<td>0.959</td>
<td>0.250</td>
<td>0.143</td>
<td>0.250</td>
<td>0.250</td>
<td>-1.490</td>
<td>13</td>
</tr>
<tr>
<td>Bin 9 (80-90%)</td>
<td>0.816</td>
<td>17</td>
<td>0.846</td>
<td>0.250</td>
<td>0.250</td>
<td>0.333</td>
<td>0.333</td>
<td>-0.863</td>
<td>13</td>
</tr>
<tr>
<td>Bin 10 (90-100%)</td>
<td>0.056</td>
<td>17</td>
<td>0.997</td>
<td>0.500</td>
<td>0.429</td>
<td>0.429</td>
<td>0.500</td>
<td>-1.712</td>
<td>13</td>
</tr>
</tbody>
</table>
Table 28: Statistics for the POA of the Rectus Femoris in Experiment 2, for swing and stance phase and by condition and arousal state. Note that there are five time windows (bins) for the swing phase (from 0-100% of the swing phase in 20% increments) and 10 time windows for the stance phase (0-100% of the stance phase in 10% increments).

<table>
<thead>
<tr>
<th></th>
<th>By Condition</th>
<th></th>
<th>By Arousal State</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>N</td>
<td>P-value</td>
<td>Medians</td>
</tr>
<tr>
<td><strong>Rectus Femoris</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Swing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bin 1 (0-20%)</td>
<td>3.750</td>
<td>18</td>
<td>0.290</td>
<td>0.542</td>
</tr>
<tr>
<td>Bin 2 (20-40%)</td>
<td>8.845</td>
<td>18</td>
<td><strong>0.031</strong></td>
<td>0.542</td>
</tr>
<tr>
<td>Bin 3 (40-60%)</td>
<td>1.776</td>
<td>18</td>
<td>0.620</td>
<td>0.458</td>
</tr>
<tr>
<td>Bin 4 (60-80%)</td>
<td>3.860</td>
<td>18</td>
<td>0.277</td>
<td>0.385</td>
</tr>
<tr>
<td>Bin 5 (80-100%)</td>
<td>3.211</td>
<td>18</td>
<td>0.360</td>
<td>0.481</td>
</tr>
<tr>
<td><strong>Stance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bin 1 (0-10%)</td>
<td>5.788</td>
<td>18</td>
<td>0.122</td>
<td>0.431</td>
</tr>
<tr>
<td>Bin 2 (10-20%)</td>
<td>3.711</td>
<td>18</td>
<td>0.294</td>
<td>0.477</td>
</tr>
<tr>
<td>Bin 3 (20-30%)</td>
<td>6.227</td>
<td>18</td>
<td>0.101</td>
<td>0.458</td>
</tr>
<tr>
<td>Bin 4 (30-40%)</td>
<td>6.810</td>
<td>18</td>
<td>0.078</td>
<td>0.458</td>
</tr>
<tr>
<td>Bin 5 (40-50%)</td>
<td>2.049</td>
<td>18</td>
<td>0.566</td>
<td>0.401</td>
</tr>
<tr>
<td>Bin 6 (50-60%)</td>
<td>1.197</td>
<td>18</td>
<td>0.754</td>
<td>0.410</td>
</tr>
<tr>
<td>Bin 7 (60-70%)</td>
<td>0.750</td>
<td>18</td>
<td>0.861</td>
<td>0.348</td>
</tr>
<tr>
<td>Bin 8 (70-80%)</td>
<td>0.260</td>
<td>18</td>
<td>0.967</td>
<td>0.415</td>
</tr>
<tr>
<td>Bin 9 (80-90%)</td>
<td>1.690</td>
<td>18</td>
<td>0.639</td>
<td>0.481</td>
</tr>
<tr>
<td>Bin 10 (90-100%)</td>
<td>3.508</td>
<td>18</td>
<td>0.320</td>
<td>0.500</td>
</tr>
</tbody>
</table>
Table 29: Statistics for the POA of the Tibialis Anterior in Experiment 2, for swing and stance phase and by condition and arousal state. Note that there are five time windows (bins) for the swing phase (from 0-100% of the swing phase in 20% increments) and 10 time windows for the stance phase (0-100% of the stance phase in 10% increments).

<table>
<thead>
<tr>
<th>Bin</th>
<th>X²</th>
<th>N</th>
<th>P-value</th>
<th>Medians</th>
<th>Z-value</th>
<th>N</th>
<th>P-value</th>
<th>Medians</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No OF</td>
<td>Random</td>
<td>Con-</td>
<td>Fast</td>
<td>Crying</td>
</tr>
<tr>
<td>Swing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bin 1</td>
<td>3.123</td>
<td>18</td>
<td>0.373</td>
<td>0.707</td>
<td>0.542</td>
<td>0.724</td>
<td>0.690</td>
<td>-0.863</td>
</tr>
<tr>
<td>Bin 2</td>
<td>5.054</td>
<td>18</td>
<td>0.168</td>
<td>0.652</td>
<td>0.542</td>
<td>0.690</td>
<td>0.708</td>
<td>-1.642</td>
</tr>
<tr>
<td>Bin 3</td>
<td>5.198</td>
<td>18</td>
<td>0.158</td>
<td>0.652</td>
<td>0.536</td>
<td>0.690</td>
<td>0.677</td>
<td>-1.083</td>
</tr>
<tr>
<td>Bin 4</td>
<td>2.486</td>
<td>18</td>
<td>0.478</td>
<td>0.450</td>
<td>0.500</td>
<td>0.500</td>
<td>0.333</td>
<td>-1.153</td>
</tr>
<tr>
<td>Bin 5</td>
<td>7.362</td>
<td>18</td>
<td>0.061</td>
<td>0.348</td>
<td>0.348</td>
<td>0.456</td>
<td>0.225</td>
<td>-1.083</td>
</tr>
<tr>
<td>Stance</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bin 1</td>
<td>9.844</td>
<td>18</td>
<td>0.020</td>
<td>0.191</td>
<td>0.279</td>
<td>0.333</td>
<td>0.028</td>
<td>-0.210</td>
</tr>
<tr>
<td>Bin 2</td>
<td>4.962</td>
<td>18</td>
<td>0.175</td>
<td>0.191</td>
<td>0.233</td>
<td>0.225</td>
<td>0.000</td>
<td>-0.267</td>
</tr>
<tr>
<td>Bin 3</td>
<td>10.947</td>
<td>18</td>
<td>0.012</td>
<td>0.211</td>
<td>0.079</td>
<td>0.101</td>
<td>0.000</td>
<td>-0.356</td>
</tr>
<tr>
<td>Bin 4</td>
<td>8.300</td>
<td>18</td>
<td>0.040</td>
<td>0.191</td>
<td>0.033</td>
<td>0.033</td>
<td>0.000</td>
<td>-0.711</td>
</tr>
<tr>
<td>Bin 5</td>
<td>4.776</td>
<td>18</td>
<td>0.189</td>
<td>0.148</td>
<td>0.033</td>
<td>0.000</td>
<td>0.031</td>
<td>-0.533</td>
</tr>
<tr>
<td>Bin 6</td>
<td>3.908</td>
<td>18</td>
<td>0.272</td>
<td>0.168</td>
<td>0.033</td>
<td>0.000</td>
<td>0.042</td>
<td>-0.178</td>
</tr>
<tr>
<td>Bin 7</td>
<td>1.873</td>
<td>18</td>
<td>0.599</td>
<td>0.214</td>
<td>0.072</td>
<td>0.138</td>
<td>0.121</td>
<td>-0.311</td>
</tr>
<tr>
<td>Bin 8</td>
<td>0.610</td>
<td>18</td>
<td>0.894</td>
<td>0.250</td>
<td>0.191</td>
<td>0.317</td>
<td>0.167</td>
<td>-0.863</td>
</tr>
<tr>
<td>Bin 9</td>
<td>1.456</td>
<td>18</td>
<td>0.692</td>
<td>0.315</td>
<td>0.273</td>
<td>0.400</td>
<td>0.333</td>
<td>-0.035</td>
</tr>
<tr>
<td>Bin 10</td>
<td>3.069</td>
<td>18</td>
<td>0.381</td>
<td>0.438</td>
<td>0.477</td>
<td>0.528</td>
<td>0.500</td>
<td>-1.363</td>
</tr>
</tbody>
</table>
Appendix E: Calculation of thresholds

Finding the alert and validation thresholds took a considerable amount of time. The first attempt utilised the Mean Amplitude Deviation (MAD), or the standard deviation of the signal from each muscle during each trial. This was multiplied with a lower and a higher constant, respectively, to determine alert and validation thresholds. However, because the amplitude and noise level (range) of the EMG signal during the trials was very different from trial to trial, it was impossible to find a constant that was suitable for each trial. Therefore, a second attempt to find suitable on-off thresholds looked back to the methodology of Spencer and Thelen (2000). These authors calculated the threshold value for EMG detection based on the modal amplitude. In their study, low base-line activity dominated, therefore the modal amplitude was ‘clearly in the noise range, whereas amplitudes much greater than the mode were clearly in the signal range’ (p. 284). The amplitude had to exceed the value corresponding with 0.15 of the modal amplitude (see Figure 56) to be considered signal, therefore the threshold was set at this value. However, the current study featured stepping trials of one minute and contained a range of activity levels, with less activity in some trials and more pervasive activity in others, and the method did not correctly identify muscle bursts.

Figure 55: From Thelen and Spencer (2000); example of a normalised amplitude histogram of one muscle (p. 284)

It was found that the differences between trials and muscles were observable in the relationship between the base-line, median and mean. Base-line level (where no muscle activity was observable and the signal consisted of mainly noise) was important to include in the threshold determination methodology; without it, the true range of the signal cannot
be determined. For trials with low activity, the median and mean did not exceed the base-line by much, whereas in high activity trials, the median and the mean were considerably higher than base-line signal. For trials with consistently high activity, the median was lower but close to the mean; for trials with sporadic defined activity, the median was closer to base-line (Figures 58, 59 and 60). The earliest threshold determination attempts found that in high activity trials, the definition between bursts was often lost and in moderate activity trials, bursts were regularly missed. In high activity trials, the alert threshold should be more conservative (higher), whereas in low and moderate activity trials, the alert threshold should include more of the signal and be less conservative (lower). Therefore, the new methodology utilised the relationship between base-line, median and mean to adjust the thresholds to the quality of the trial (high, moderate or low activity).

Base-line amplitude was determined from the signal by calculating the mean over a window of five seconds in each muscle in which the amplitude of the signal was at its lowest and stable (no peaks). In very high activity trials, the window size was reduced to minimally three seconds. If no baseline could be established, or no clear signal was evident, the trial was not used. To determine the alert threshold, a value was extrapolated on a fictive line between baseline, median and mean values, with increments of one unit on the x-axis. To determine the validation threshold, an extrapolation on a fictive line between the baseline and the mean was used. Because the trend line of the alert threshold was based on three values, the slope of this line was shallower, resulting in a lower value for the alert threshold than for the validation threshold (Figure 57). The size of the median value (high for high activity trials and low for low activity trials) adjusted the slope inclination to give more conservative thresholds when there was more activity during the trial, and less conservative thresholds with less activity (Figure 57).
Testing showed that both alert and validation thresholds were on the low side when the signal-to-noise-ratio (SNR) was low (low muscle burst amplitudes with high baseline levels), including too many small peaks in the noise. Therefore, the extrapolation factor was based on the inverted signal-to-noise ratio (baseline divided by the mean, between 0 and 1), ensuring that when the SNR was low, thresholds were adjusted upwards.

Overall, the formula used to obtain the alert threshold was:

$$\text{Alert Threshold} = \text{Slope BMM} \times \text{SNR} + \text{Intercept BMM}$$

with BMM as a fictive line through the baseline, median and mean. The validation threshold was calculated with the following formula:

$$\text{Validation Threshold} = \text{Slope BM} \times \text{SNR} + \text{Intercept BM}$$

with BM as the fictive line through the baseline and mean.
Comparing this method to visual coding of 84 muscles showed that the method was reliable, with a percentage agreement of 85.4%.

Figure 57: Example of baseline, median and mean levels for a low activity trial
Figure 58: Example of baseline, median and mean levels for a moderate activity trial
Figure 59: Example of baseline, median and mean levels for a high activity trial
Appendix F: Example of statistical analysis

First, the four assumptions were tested (normality, homogeneity, linearity and independence) for all data. The majority of data were not normally distributed, therefore nonparametric tests (Friedman tests) were used.

### Descriptive Statistics

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25th</td>
<td>50th</td>
<td>75th</td>
</tr>
<tr>
<td>MovC1</td>
<td>20</td>
<td>.4692301</td>
<td>.20899060</td>
<td>.11246</td>
<td>.72447</td>
<td>.6671967</td>
</tr>
<tr>
<td>MovC2</td>
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<td>.24389461</td>
<td>.09174</td>
<td>.90589</td>
<td>.5832102</td>
</tr>
<tr>
<td>MovC3</td>
<td>20</td>
<td>.4247733</td>
<td>.23078246</td>
<td>.08466</td>
<td>.79403</td>
<td>.6301706</td>
</tr>
<tr>
<td>MovC4</td>
<td>20</td>
<td>.3944374</td>
<td>.22533154</td>
<td>.07887</td>
<td>.74755</td>
<td>.5907673</td>
</tr>
</tbody>
</table>

When a significant difference was found, *post hoc* analysis was performed through a Wilcoxon Signed-rank test.

### Test Statistics

<table>
<thead>
<tr>
<th></th>
<th>MovC2 - MovC1</th>
<th>MovC3 - MovC1</th>
<th>MovC4 - MovC1</th>
<th>MovC3 - MovC2</th>
<th>MovC4 - MovC2</th>
<th>MovC4 - MovC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>-.672&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-1.045&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-2.240&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-.336&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.896&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-1.307&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Asymp. Sig. (2-tailed)</td>
<td>.502</td>
<td>.296</td>
<td>.025</td>
<td>.737</td>
<td>.370</td>
<td>.191</td>
</tr>
</tbody>
</table>

<sup>a</sup> Wilcoxon Signed Ranks Test

<sup>b</sup> Based on positive ranks.
To adjust the alpha level for multiple comparisons, Bonferroni-Holm corrections were made.

<table>
<thead>
<tr>
<th>Z</th>
<th>Asymp. Sig. (2-tailed)</th>
<th>m (number of p-values)</th>
<th>corrected alpha</th>
<th>P&lt;corrected alpha?</th>
</tr>
</thead>
<tbody>
<tr>
<td>MovC4 - MovC1</td>
<td>-2.240 b</td>
<td>.025</td>
<td>6</td>
<td>0.0083</td>
</tr>
<tr>
<td>MovC4 - MovC3</td>
<td>-1.307 b</td>
<td>.191</td>
<td>5</td>
<td>0.0100</td>
</tr>
<tr>
<td>MovC3 - MovC1</td>
<td>-1.045 b</td>
<td>.296</td>
<td>4</td>
<td>0.0125</td>
</tr>
<tr>
<td>MovC4 - MovC2</td>
<td>-.896 b</td>
<td>.370</td>
<td>3</td>
<td>0.0167</td>
</tr>
<tr>
<td>MovC2 - MovC1</td>
<td>-.672 b</td>
<td>.502</td>
<td>2</td>
<td>0.0250</td>
</tr>
<tr>
<td>MovC3 - MovC2</td>
<td>-.336 b</td>
<td>.737</td>
<td>1</td>
<td>0.0500</td>
</tr>
</tbody>
</table>

In this particular case, a significant difference was found, although the difference was not significant after Bonferroni-Holm correction for multiple comparisons.
Appendix G1: Ethics decision

Le comité a été saisi le 04/02/2014
par le promoteur : CNRS
d’une demande d’avis pour le projet de recherche :
N° : CNRS protocole 06010 : « Vision et Locomotion chez le Nourrisson » -
« Vision and Locomotion in Infants »
dont l’investigateur coordonnateur est le Pr. Dominique CABROL.

Le Comité a examiné la demande de modification substantielle n° 4 du 04/02/2014 au protocole -version 5 du 04/02/2014-, la note d’information et de consentement du patient - version 5 du 04/02/2014-, la liste des Investigateurs -version 5 du 04/02/2014- (n° promoteur) - l’amendement n° 4 (n° CPP) - daté du 04/02/2014.

Lors de sa séance du 04 MARS 2014
Conformément aux dispositions de l’article R1123-11 du Code de la Santé Publique, ont participé à la délibération :

<table>
<thead>
<tr>
<th>Prénom(s)</th>
<th>Comité</th>
<th>Suppléant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pierre-Emmanuel</td>
<td>Personne modifiée en matière de recherche biomédicale</td>
<td>Pr. Rémy DROUET</td>
</tr>
<tr>
<td>Bruno BERNARD</td>
<td>Médicale générale</td>
<td>Dr. Bernard WEIL</td>
</tr>
<tr>
<td>Louis LOUBERGE</td>
<td>Pharmacienne hospitalière</td>
<td>Dr. Nael ZAIR</td>
</tr>
<tr>
<td>Anne GIRAUD</td>
<td>Informateur</td>
<td>(conseiller en matière bioclinique)</td>
</tr>
</tbody>
</table>

Motivations du comité: l’amendement ne modifiant pas la balance bénéfices-risques est bien explicité.

Le Comité rend un AVIS FAVORABLE

Commentaire : néant.

Nature des modifications : changement d’investigateur coordonnateur de l’étude, le Pr. D. Cabrol est remplacé par le Pr. François GOFFINET ; le laboratoire de M. Barbu-Roth a changé de numéro d’unité UMR 8156 à UMR 8242.

Signé : Le Président
David SIMHON
Chers parents,

Votre enfant est encore bien petit, et pourtant, il est déjà lancé dans la découverte du monde qui l’entoure. Pendant sa première année, sa motricité et sa perception se développent plus vite que pendant toute autre période de sa vie. De jour en jour, vous assistez à ses progrès dans le regard, la tonicité musculaire, le maintien de la posture, la coordination des mouvements des bras et des jambes. D’autres progrès sont plus cachés, et pourtant impressionnants. Savez-vous par exemple que, dès la naissance, votre enfant est non seulement capable de marcher, mais d’adapter le rythme de ses pas aux mouvements de son environnement visuel et à la perception de différents sons ou odeurs ?

Cette faculté étonnante a été découverte grâce aux recherches menées par des scientifiques pour explorer les capacités précoces des nourrissons : elle ouvre des perspectives totalement nouvelles pour comprendre comment le nourrisson apprend à coordonner ses mouvements avec ceux de son monde extérieur.

Afin de savoir quels sont les stimuli les plus appropriés pour permettre au nourrisson de synchroniser au mieux ses mouvements avec son environnement, nous souhaiterions vous inviter avec votre enfant, à participer à une étude que nous menons dans le service du Pr. Goffinet de la Maternité du Groupe Hospitalier Cochin-Port Royal-Saint Vincent de Paul-La Roche Guyon. Cette étude, intitulée « vision et locomotion chez le nourrisson », est menée par Marianne Barbu-Roth (Docteur en Neurosciences, chargée de recherche au CNRS), Viviane Huet (Technicienne à l’Université Paris Descartes) et Vincent Forma (Doctorant) dans le laboratoire Psychologie de la Perception du CNRS et de l’Université Paris Descartes, avec l’aide de Joëlle Provasi (Ecole Pratiques des Hautes Etudes), Caroline Teulier (Université d’Orsay) et Kim Siekerman (Université de Limerick).

Votre participation consiste à venir, une seule fois, accompagnés de votre enfant, au babylab (Laboratoire de Sciences Cognitives et Psycholinguistiques) au niveau-1 de la Maternité. Votre enfant participera en votre présence à une évaluation de 15 minutes, spécialement conçue pour les nourrissons. Cette évaluation est basée sur la mesure du nombre de pas de l’enfant en réponse à la détection d’images mobiles. Dans certains cas, des odeurs et des sons pourront être ajoutés. Chaque enfant est placé dans les bras d’une expérimentatrice, en position érigée au-dessus d’une table ou d’un petit tapis roulant, ou en position ventrale (avec ou sans l’aide d’un support en mousse placé sous son abdomen). Nous enregistrerons les mouvements et parfois l’activité musculaire des jambes et des bras de l’enfant en réaction aux mouvements qu’il détecte sur la table (l’activité musculaire est mesurée par 4 capteurs placés sur chaque jambe de l’enfant). En général, les enfants participent sans problème à ces évaluations, mais si votre enfant ne se sent pas à l’aise, nous interrompions immédiatement la séance.

Afin de mieux analyser les réponses des enfants, nous aurons besoin d’enregistrer les sessions sur vidéo. Bien entendu, ces données resteront strictement confidentielles et anonymes, et seuls les membres de l’équipe de recherche pourront y avoir accès : aucun nom propre ne figurera sur les enregistrements et ceux-ci seront gardés dans des armoires fermées à clé. Si vous le souhaitez, les enregistrements vidéo pourront être détruits après l’exploitation qui en sera faite dans le cadre de cette recherche.

Bien entendu, la participation ou non de votre enfant à ce projet ne peut en aucun cas modifier vos relations avec le service de la Maternité du Groupe Hospitalier Cochin-Port Royal-Saint Vincent de Paul-La Roche Guyon. Si vous souhaitez des renseignements supplémentaires, vous pouvez contacter Marianne Barbu-Roth (06 1861 0915), Vincent Forma (06 23 40 14 37) ou Viviane Huet (01 4286 4230).

Avec tous nos remerciements pour votre collaboration,

Marianne Barbu-Roth
Appendix G3: Consent form

Groupe Hospitalier Cochin-Port Royal – APHP
Protocole N° 06010 – Laboratoire Psychologie de la Perception UMR 8242 CNRS-Université Paris Descartes

CONSENTEMENT DE PARTICIPATION
pour une recherche sans bénéfice individuel direct
(formulaire de consentement concernant les représentants légaux d’un sujet mineur)

Nous soussignés :

déclarons accepter que l’enfant …………………………………… dont nous avons la charge légale participe à la recherche biomédicale intitulée « Vision et Locomotion chez le Nourrisson » organisée par le Professeur François Goffinet et Marianne Barbu-Roth, chargée de recherche au CNRS dans les conditions précisées ci-dessous et pour laquelle le CPP Ile de France 3 a émis un avis favorable le 25/04/2006 et renouvelé son avis les 22/9/2009, 7/6/2011 et 13/12/2012.

Le CNRS promoteur de la recherche a, conformément à la loi, contractée une assurance.

Nous avons bien reçu les informations incluses dans la feuille ci-jointe.

Il nous a été précisé que :

- Nous sommes libres d’accepter ou de refuser ainsi que d’arrêter à tout moment la participation de l’enfant dont nous avons la charge, à la recherche.

- Les données qui concernent l’enfant dont nous avons la charge resteront strictement confidentielles. Nous n’autorisons leur consultation que par des personnes qui collaborent avec Marianne Barbu-Roth et, éventuellement, un représentant des autorités de santé.

- Nous pourrons à tout moment demander des informations à Marianne Barbu-Roth ou à l’un des investigateurs conjoints (Vincent Forma, Joëlle Provasi, Viviane Huet, Kim Siekerman, Caroline Teullier).

- La publication des résultats ne comportera aucun résultat individuel identifiant.

- Cette recherche est sans bénéfice individuel pour les personnes qui y participent.

- L’enfant dont nous avons la charge ne pourra pas participer à une autre recherche sans bénéfice individuel direct pendant une période de 0 jour, après la fin de l’étude.

- Si nous le désirons, nous pouvons être tenus au courant des résultats globaux de la recherche en nous adressant à Marianne Barbu-Roth ou à l’un des investigateurs conjoints (Vincent Forma, Joëlle Provasi, Viviane Huet, Kim Siekerman, Caroline Teullier).

- Notre consentement ne décharge pas les organisateurs de la recherche de leurs responsabilités.

- Nous conservons tous nos droits garantis par la loi.

Fait à ……………., le ……….

Signature de la mère et/ou du père

Nom et signature de l’investigateur

M. Barbu-Roth

F. Goffinet

G.H. COQHIN - BROCA - HOTEL DI
Majenient, Port Royal
Chef de la Sec
RFFC : 1 000 00 77 746
53, Avenue de l’Observatoire
75670 PARIS CEDEX 14
Tel : 01 56 41 38 00 - Fax : 01 58 41 38 91

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Appendix H: Published Article

Article first published online in Wiley Online Library (wileyonlinelibrary.com).
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Developmental Psychology
2015, 57 (2), pp. 247-254
Treadmill stimulation improves newborn stepping
Sikerman, K, Barbu-Roth, M, Anderson, DI, Donnelly, A Goffinet, F Teulier, C
http://dx.doi.org/10.1002/dev.21270