AFFECTIVE TOUCH

IN INFANCY

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ORIGINALITY STATEMENT

'I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at the University of London or any other educational institution, except where due acknowledgment is made in the thesis. Any contribution made to the research by others, with whom I have worked at University of London or elsewhere, is explicitly acknowledged in the thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.'

Laura Pirazzoli, 19th of October 2018

The following research thesis includes work that appears in the following articles:

Pirazzoli, L., Lloyd Fox, S., Braukmann, R., Johnson, M. H., & Gliga, T. (2018). Hand or spoon? Exploring the neural basis of affective touch in 5-month-old infants. *Developmental Cognitive Neuroscience*.

The work is cited in the relevant chapters accordingly.

ABSTRACT

Social touch is ubiquitous in caregiver-infant interactions. Research on animal models and preterm human infants has shown that touch is critical for a young organism's physical and psychological growth. However, the role that social interaction through touch plays in the development of typically developing human infants is poorly understood. The research presented in this thesis investigated neural specialization for social touch and the mechanisms through which social touch might promote early development. I focus on a particular type of touch, slow velocity stroking, shown to activate a particular type of skin fibers in human adults, the CT-fibers, and to elicit affective responses (henceforth affective touch). Research presented here investigated cortical activation and autonomic responses to affective touch, during the first year of life.

Firstly, in experiments 1 through 4 functional Near Infrared Spectroscopy (fNIRS) was employed to measure haemodynamic responses to affective and non-affective touch over inferior frontal and temporal cortices. Experiments 1, 2 and 3 used three different non-affective stimuli and revealed that specialization to affective touch in key nodes of the social brain has not developed yet in 5 to 7-months-old infants. Results from Experiment 4 suggest that this specialization emerges near the end of the first year of life (10-month-olds).

Secondly, in experiments 5 and 6 heart rate changes to affective and non-affective touch were measured in three different age-groups (2, 7 and 9-month-old). Results revealed that infants in neither group displayed differential responses to the touch stimuli. Further, experiment 5 explored whether affective touch modulates visual attention but an effect was not found.

Taken together these findings showed that preferential processing of affective touch is not evident during early development, at least when investigating neural and autonomic responses. In all my studies, I strived to present tactile stimuli in the absence of other social cues, thus ensuring that any effects would have been specific to touch. In the final discussion I suggest that the lack of context might have prevented infants from identifying affective touch. I also discuss the possibility that other forms of inter-personal touch, and not CT-targeted touch, may be critical in early human development, and should be investigated in future research.

ACKNOWLEDGMENTS

And finally, I get to write the acknowledgements. I drafted this bit countless times in my mind while cycling through Hyde Park on my way to work.

The main thanks are for my supervisors Mark, Teea and Sarah. Thanks for guiding and supporting me throughout the 'adventures of studying affective touch' and, while doing so, for allowing me a glimpse into the greatness of your minds. Often, after one of our Tuesday meetings I would tell my family how honored I was to be able to sit in the same room with you all. I used to say that my mind was so happy to witness and take part in your flow of thought and reasoning that it felt like it left my body and soared with joy in Marks' office (I admit that in Italian it sounded better! ☺).

Thanks for guiding me with patience and for never making me feel stupid (even when I didn't have the brightest of ideas or repeated silly mistakes again and again and again). A special thank goes to Teea, who taught me so much and more than anyone has shaped my critical thinking. With you I learnt how to ask the right questions and that all findings are interesting.

Equally important thanks are for my friends and family who kept me sane during my adventures in affective touch.

I approached the PhD experience like Murakami approached his first full length marathon, running the original course from Athens to Marathon. Like Murakami I was totally unprepared for what actually lay ahead of me. He thought that the worst would have been the distance which he had never covered before, and instead found that the unbearable heat of Greek summers and the traffic on the highway were the hardest obstacles (Murakami, 2008, *what I talk about when I talk about running*). In a similar way, when I started I was worried about the length of the PhD, and little did I know that programming, writing in concise scientific English, responses to the toothbrush and imposter syndrome were actually going to pave my way along this experience. However, I am thankful for having pursued this journey until the end because along with the rollercoaster came growth and a whole range of wonderful human experiences. Indeed, unlike Murakami, who was accompanied on his journey by only two people in a van that only provided him with water upon request, I could count on a bigger team that provided me with more than water. So, my wholeheartedly felt thanks go to anyone who during this journey helped me to stay hydrated, both mentally and physically.

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LIST OF ABBREVIATIONS

ACTH	adrenocorticotropic hormone
ANS	autonomic nervous system
AV	atrioventricular (node)
DOT	diffusion optical tomography
DPF	differential pathlength factor
DR	defensive reflex
ECG	electrocardiogram
EDA	electrodermal activity
EEG	electroencelography
fMRI	functional magnetic resonance imaging
GH	growth hormone
GR	glucocorticoid receptor
HbO$_2$	oxy-hemoglobin
HHb	deoxy-hemoglobin
HPA	hypothalamic-pituitary-adrenal
HR	heart rate
HRV	heart rate variability
IBI	inter-beat interval
IFG	inferior frontal gyrus
ITG	inferior temporal gyrus
KMC	kangaroo mother care
LC	locus coeruleus
LG	licking and grooming
LMM	linear mixed model
LTM	low-threshold mechanoreceptor
MEG	magnetoelectroencephalography
MS	maternal separation
MTG	middle temporal gyrus
NE	norepinephrine
NICU	neonatal intensive care units

NIRS	near infrared spectroscopy
ODC	ornithine decarboxylase
OFC	orbitofrontal cortex
OR	orienting reflex
RSA	respiratory sinus arrhythmia
SA	sinoatrial (node)
SF	still face (paradigm)
STS	superior temporal sulcus
TMS	transcranial magnetic stimulation
TPJ	temporoparietal junction

Chapter 1

General Introduction

Intuitively, we accept that the sense of touch is crucial for human development: both across mammals and humans it is impossible to imagine a mother and her young not contacting each other. A substantial volume of (mostly behavioural) research run both under experimental and naturalistic conditions has compellingly shown that the amount of interpersonal touch experienced early in life is related to positive developmental outcomes. It was advanced that social touch supports physiological and emotional regulation and that it promotes social interactions and communication. To further strengthen the idea that touch plays a crucial role in development, when researchers turned to those situations where the infant's experience of social touch is altered or disrupted they consistently reported detrimental effects. For example, maternal depression, which alters several elements of typical mother-infant interactions, including the quality of maternal touch, has been used as a model for altered tactile experience. The fact that some of the effects associated with postnatal depression (i.e. high negative emotionality and stress reactivity) are reversed when specific types of social touch are implemented in the infants' experience (skin stroking vs. passive body contact and massaging vs. rocking) lends support to the idea that touch subserves a regulatory function (Sharp et al., 2012; Field et al., 1996). Institutionalised care has also been indicated as a model for early tactile deprivation.

The critical role of interpersonal touch for social and cognitive development has been accepted without debate; the idea naturally emerged that among other modalities involved in mother-infant interaction, touch had a pivotal role. However, while it is clear that interactions involving touch positively impact development, the underlying mechanisms have not yet been revealed and it is not clear to what extent social touch on its own contributes to the reported outcomes. While evidence coming from naturalistic models of social touch deprivation is precious to inform our understanding of the functions of social touch, it still does not allow us to uniquely link touch to these outcomes. Indeed, both in the context of maternal depression and of institutional care, many key elements of the mother-infant relationship other than touch are missing. Given the impossibility of measuring the effects that a pure lack of social touch has on development, **in this PhD I took an**

experimental approach in which I manipulated the amount and type of social touch and observed its effects on behavior and physiology.

An interesting point that emerges from evidence that linked touch to positive developmental outcomes is that infants do not perceive all social touch equally and that it is not the total sum of all somatosensory experiences that matters for development. Instead, it is a defined group of social interactions mediated by touch that seems to be critical for development to occur along a typical trajectory. Therefore, the first question faced by those investigating social touch in early development is one that concerns the stimulus itself: *what defines social touch*? Out of the myriad of touches that are typically observed as part of parent-infant interactions, how can we identify social touch that is important for development? The discovery of low-level mechanisms (C-tactile - *CT*- afferents in the skin) dedicated to the processing of caress like touch has fuelled a new area of research focussed on touches that maximally activate these fibers. In adults stimulation of these afferents through gentle stroking of the skin was associated with activation of the social brain. This new area of research supports the idea that not all social touch contributes to development in the same way and that gentle stroking could hold a prominent role. However, the developmental origins of this sensitivity to CT touch are currently unknown and developmental studies are needed to understand to what extent specificity to skin stroking is experience dependent. In order to gather evidence that can further our understanding of this issue, gentle skin stroking has been used as social touch across the experiments presented in this thesis with infants in five different age groups (between 1 and 12 months of age). Thus, **for the purpose of this thesis social touch was defined as gentle skin stroking.** Once the social touch stimulus is defined at the researchers' end, we want to ask how infants identify social touch within the more general tactile stimulation they are exposed to. One possibility is that discrimination is driven by the physical properties of the different touches. **To this end over the six experiments presented in this thesis I explored different touch contrasts in which the physical properties of non-social touch were manipulated.**
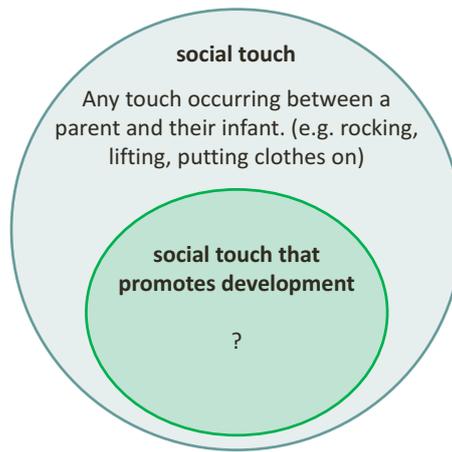
Figure 1.1 Diagram representing the first question of this thesis: what defines social touch.

A following question is that of '*how does social touch promote development*?'. Can we identify in the organism's specific response to social touch elements that could be responsible for such effects? A mechanistic approach to the study of social touch is necessary in order to reveal to what extent this early life experience contributes to development. Once questions regarding the stimulus are resolved and mechanisms pinpointed, research of models of atypical development where processing of interpersonal touch is altered, such as ASD, will build on these findings to potentially devise targeted early interventions.

It was suggested that one of the functions for the early development of the sense of touch could be that of scaffolding the development of the social brain (for a review see McGlone et al. 2014). Given that touch is the first sensory modality to develop prenatally (both anatomically and functionally), it was posited that CT afferents are stimulated as early as in utero (via massaging exerted by the amniotic fluid) potentially providing the developing social brain with its primary template. Stimulation of these afferents continues through early postnatal development, a time when episodes of gentle touch abound. According to this theoretical perspective cortical specialization to social touch at key nodes of the social brain should emerge early in development. **This motivated the first set of experiments presented in this thesis where I measured the neural underpinnings of social touch.**

Evidence from both animal and human research has suggested that another function subserved by social touch is that of regulating the autonomic nervous system increasing parasympathetic activity. If infants are sensitive to and discriminate CT touch at the autonomic level, this could offer a mechanism mediating some of the reported effects of touch on emotional and physiological regulation. **This motivated a second set of experiments presented in this thesis where I measured how social touch modulates heart rate responses.**

The current thesis is structured as follows:

Chapter 1 provides the reader with a general introduction to the current state of knowledge on social touch processing in infancy. In this chapter, before dissecting the specific contribution(s) of social touch on human development I first turn my attention to the animal kingdom. Indeed, over five decades of animal research answered the questions just raised ('what defines social touch' and 'how does social touch promote development'). Experiments with animal models (mainly rodents) which allow for precise control over experimental variables, offer the opportunity to establish causal relationships between maternal touch and the pup's behaviour and to explore underlying mechanisms. A comparison between animal models and humans can be attempted due to the fact that similarly to humans, most of the mother-pup interactions are also mediated by social touch. Since the experience of this social somatosensory input early in life is shared with our ancestors, it is reasonable to think that some of its effects may be conserved in our own species. Given the scarcity of research on the role of social touch in human early development findings in animal models serve as a precious starting point to formulate questions and tentative hypotheses. If results across mammalian species align to one another we could infer that tactile behaviours and the mechanisms through which touch affects development have been conserved throughout evolution.

It is crucial that a number of limitations are kept in mind when drawing comparisons across species and caution should always prevail. For example, the repertoire of touches available to human mothers is far more extended than that of rodent dams. Further, these touches, even when similar in their physical

characteristics across species, are received by infants that present large differences in the stage and rate of development of their nervous system (e.g., rodents, contrary to humans, do not develop their senses of vision and hearing until the second postnatal week).

This seemingly long detour into animal research is therefore important for several reasons. Given the paucity of studies in humans, understanding the findings that emerged from the large body of work on animal models holds the potential to guide new research and help us understand to what degree the function(s) of touch remained conserved throughout evolution. Furthermore, animal works are often cited to lend support to statements on the importance of touch for early human development. In order to understand to what extent this extrapolation is justified a comprehensive account of the animal research is needed. One of the aims of this initial overview is to provide the reader with tools to make this evaluation.

Following the section on animal research I review the literature on the effects of social touch on a human model of early tactile deprivation, preterm birth, and draw comparisons between findings in animal and human infants. I then move on to present evidence from typically developing, born at term infants. This section is divided into studies that investigated behavioural, brain, autonomic and endocrine responses to social touch. Trying to tie together findings from these different lines of work, I conclude with a consideration on the challenges of defining social touch and suggest a possible way of reframing this question.

The next section introduces the CT system together with the available evidence of sensitivity to CT touch (gentle skin stroking) early in life. I explain why for this thesis social touch was defined as CT touch. At the end of Chapter 1 I provide an outline of the experiments carried out for this PhD and advance specific hypotheses for each.

Chapter 2 describes the methods employed to investigate the mechanisms that mediate the posited effects of social touch on development: ECG, fNIRS and eye-tracking.

Chapters 3 and 4 use fNIRS to investigate mechanisms at the cortical level and test the hypothesis that one possible mediator of the beneficial effects of development is an early cortical specialization to social touch in key nodes of

the social brain. This could support the reported effects on socio-emotional development.

 Chapters 5 and 6 use ECG to measure mechanisms at the autonomic level. The finding that social touch decreases heart rate could support the reported regulatory function of touch. Chapter 5 also tests the hypothesis that an increase in parasympathetic activity is linked to a state of focused attention (measured as changes in visual attention) which is optimal for learning. While learning was not directly tested, revealing a link between social touch, heart rate decreases and focused attention could support the effects on cognitive development attributed to social touch.

 Chapter 7 closes this thesis with considerations on the findings that emerged from the different experiments in light of questions raised in Chapter 1 and of the hypotheses advanced. New potential avenues for research in this field are outlined.

 With the work in this thesis, I hope to shed light on why it is important that we ask what the specific contributions of social touch on development are. Until we gain this understanding, claims on the pivotal role that social touch plays in promoting development should not be accepted without questioning. Throughout my experiments I aim to reveal whether and how infants process social touch, defined as CT touch, when this is presented in isolation. I aim to understand whether sensitivity to social touch is experience dependent or whether it can be measured early in development, when extensive experience of this stimulus has not yet occurred. While the present body of work is certainly important, given the current state of knowledge we have on this topic, it cannot be considered exhaustive. It represents an initial step in the right direction. Social touch encompasses a near infinite range of interpersonal tactile stimulations and whether or not I show that infants are sensitive to the specific stimulus I chose, researchers should continue to spend time asking themselves 'what defines social touch?' and to explore what defines the borders that separate social touches important for development from more general tactile stimulation. These borders are now blurred and hard to see, but revealing them and drawing clear marks represents in my opinion a rather interesting challenge for developmental research to tackle in the near future.

1.1 Touch in animal models

In this first section I will provide an overview of the major findings on the effects of touch on early development, focusing first on studies with macaque monkeys and then on rodent studies. While non-human primates offered a fascinating opportunity to measure how touch impacts behavioural development (in a species phylogenetically closer to us), rodents have been essential for the study of underlying mechanisms. The history of scientific interest in early tactile stimulation dates back to the 1950s, when researchers first became aware of the importance of touch in animal models. However, the serendipitous timing of some of the studies undertaken does not reflect a joint effort towards answering the same questions through different species as different research programs were carried out independently and were motivated by different questions.

1.1.1 The importance of 'contact comfort' in rhesus macaques

Harry F. Harlow was the first to show how crucial the sense of touch is for development and to highlight the consequences of its deprivation early in life. With his research, Harlow wanted to investigate the development of the affectionate response observed between the human infant and her mother. Specifically, he wanted to identify the critical factors for the formation of this affectionate bond. Given the limitations imposed by studying human infants, who at birth only exhibit a limited repertoire of responses, he turned to macaque monkeys. Macaque monkeys, like humans, also form long lasting affectionate attachments with their mothers but their level of maturation at birth allows the measurement of *affective* reactions as early as the first few days of life. A two-day-old rhesus macaque can already move around independently, and actively explore the environment, allowing researchers to test affectionate responses (e.g. through measuring preference between two objects or behaviours in an open field test) much earlier than would be possible in humans with this approach.

The idea that 'tactile contact' is a variable essential to the early affective responses was informed by observations Harlow made while raising infant rhesus monkeys that had been separated from their mothers, in his primate laboratory, at the University of Wisconsin. Indeed, he noticed that infant macaques showed strong attachments to the cloth pads that covered the floor of their cages; when these were removed to be replaced, they responded with long lasting, violent bouts of negative emotionality. The studies on affection that followed are amongst the most famous in the history of psychology.

To manipulate the variable of tactile contact he devised two surrogate mothers, one covered in terry cloth and known as 'the cloth mother' and one made of wire, hence referred to as 'the wire mother'. In one of his famous experiments, newborn macaques were placed in a cage with the two surrogates, where the wire mother provided food while the cloth mother did not. Surprisingly he observed that the baby macaque monkeys spent the majority of their time on the cloth mother, even though it didn't provide them with nourishment (Harlow & Zimmermann, 1959; Harlow, 1958). Harlow concluded that what he calls 'contact comfort' is a variable of overwhelming importance for the formation of affectionate responses whereas nursing plays a negligible role. In another experiment, macaques raised only by the wire mother suffered from diarrhoea more often and had troubles digesting their milk as compared to their peers who had been raised by the cloth mother. These results suggested that the lack of social contact early in life is an extremely stressful event.

Furthermore, Harlow showed that the contact was important not only during early development but also as the infant macaques got older. Indeed, being assigned to one or the other surrogate influenced older monkeys' behaviour during an open-field test. In the presence of the mother, those monkeys raised with single cloth mothers showed positive responses to her making frequent contact, showed high levels of exploration and low levels of negative emotionality. On the contrary, for those monkeys raised with single wire mothers, the presence of the surrogate did not reduce their emotionality

and they spent little time both in contact with her and in exploration of the new environment[1] (Harlow & Zimmermann, 1959).

What surprised Harlow the most was to find out that contact comfort plays a more important role than nursing for the development of the mother-infant bond. Such an unexpected finding led him to propose that one of the functions of nursing is actually that of ensuring frequent contact between the dyad (Harlow, 1958); in 'contact comfort' he identified 'the nature of love', as he titled his first paper on the topic in 1958. Harlow did not initially aim to study touch per se as he was interested in the theoretical framework of attachment and wanted to complement Bowlby's early theories with experimental data. In doing so he discovered that there is no attachment without social touch. This series of experiments was followed by studies aimed at exploring other variables involved in the modulation of the mother-infant tie, such as rocking and clinging. Overall, these studies were the first to suggest that touch may also be very important in human babies.

In another series of rather controversial studies, Harlow showed the devastating consequences that the total absence of social contact from birth has on the macaques (Harlow & Harlow, 1971). Macaque monkeys raised without their mother or without a surrogate, in isolation chambers, started making repeated contact with their own body in the form of self-clasping, rocking and huddling (Harlow, et al., 1965, 1966; Harlow & Harlow, 1962;). As adults, when these monkeys were exposed to conspecifics, they had impaired social and exploratory behaviours (e.g. they did not engage in grooming behaviours). Additionally, when isolate-reared females were artificially inseminated, they were not capable of taking care of their offspring. In follow-up studies Harlow showed that contact, later in life, could reverse the effects of early deprivation. Indeed, exposing socially isolated 6-months-old[2] macaques to young macaques

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[1] This work provided the basis for Bowlby's conceptualization of the 'secure base' in the context of his theory of attachment (Bowlby, 1969).

[2] Six months of isolation had been shown to be sufficient to have profound and permanent social deficits in previous studies (Harlow, Dodsworth, & Harlow, 1965; Harlow & Harlow, 1962; Harlow, Harlow, Dodsworth, & Arling, 1966; Mason, 1963; Rowland, 1964; Sackett, 1968a; Senko, 1966).

in the clinging stage of development led to recovery of all the behavioural deficits caused by the isolation rearing (Suomi & Harlow, 1972).

The studies presented thus far measured the effects that early exposure to social touch (and the lack thereof) has on macaque monkeys' behaviour, in controlled laboratory settings. One attempt to go beyond behavioural measures is represented by the work of Stephen Suomi (former student of Harry F. Harlow) in the field of psychoneuroimmunology. Suomi and his group studied how early experiences affect the stress response and the immunological system. As pertains to social touch, they investigated whether touch experienced early in life in a naturalistic setting[3] can impact the strength of the immune response. Findings show that the amount of social contact and grooming received during the first 6 months of life predicts the immune response at 1 year of age (measured as the antibody response following a tetanus inoculation) (Laudenslager et al., 1993), pointing to a link between touch and health related outcomes.

Taken together these findings were the first to suggest that the early experience of social touch, in the form of passive contact with the mother, has long-lasting multidimensional impact on the macaque's development. Thus, despite Harlow's original interest for the mother-infant bond, his findings had broader implications, shedding a light on the nature of environmental factors important for infant development. This series of experiments showed for the first time the importance of social tactile contact for infants (of this mammalian species at least), and it is now rare not to find them mentioned in books and papers about social touch in development.

Returning to the two questions that opened this thesis, work on rhesus macaques cannot help us answer the question concerning mechanisms (*how does social touch promote development*?) since it has only measured behaviour. However, this work shows that in this mammalian species, passive body contact with a conspecific is a form of social touch important for development, providing a partial answer to the second question (*what defines social touch?*). We do not know whether this is the only form of social touch important for

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[3] An island in Puerto Rico where the Carribbean Primate Research Center is located.

macaque monkeys' development since other forms tactile interactions (e.g. grooming) have not been explored.

1.1.2 The lifelong effects of licking and grooming in rodent models

1.1.2.1 From "gentling" to handling

The Harlow studies showed us how touch impacts behavioural development but did not investigate the mechanisms behind these effects. Understanding of such mechanisms was reached with studies on handling in rats and mice. Work on touch in rodent models was unrelated to Harlow's research and unlike it, had no roots in attachment. The theoretical framework within which these studies took place was that of understanding the long-term impact of early life experiences. The choice of measuring the effects of touch in particular was initially motivated by some serendipitous observations made across rodents' labs: *animals that were touched frequently by the researchers behaved differently from those touched less frequently.*

Almost one-hundred years ago, the first to share his observations with the scientific community was the anatomist Frederick S. Hammett, of the Wistar Institute of Anatomy in Philadelphia. Hammett reported that albino rats that were "petted and gentled" were less timid, more relaxed and had higher survival rates following a thyroidectomy procedure, compared to animals not exposed to petting and gentling (Hammett, 1922). A few years later, as evidence on the effects of petting on the rats' behaviour was accumulating, this procedure was implemented as a standard practice at the Wistar Institute and it was claimed that "individual attention [to rats], shown by handling and petting, is essential for securing uniform reactions when used as research animals'' (Greenman & Duhring, 1931).

Following the findings at the Wistar Institute, a new area of research grew that aimed at quantifying and understanding the effects of handling. Different groups started manipulating handling as the independent variable in their experiments and reporting its effects on emotional reactivity, learning and the stress response. For example, in Otto Weininger's work, rats were divided into two groups, one received no extra handling and the other one received

extra handling in the form of 'gentling'. Gentling consisted of removing the rats from the cage and stroking them on the back for 10 minutes a day, for 3 weeks post weaning. At later time points rats were either tested in in an open field situation and their emotional reactivity was measured, or they underwent a stressful event (food and water deprivation) then sacrificed, and the weight of their adrenals was used as a measure of their stress response. Heavier adrenals would index greater adrenocorticotropic hormone (ACTH) secretion from the pituitary gland (see footnote 5 for a description of hypothalamic-pituitary-adrenal HPA responses to stress). Gentled rats had greater mean weight (immediately after the 3 weeks of gentling and also during adulthood), they showed reduced emotional reactivity (more activity and less freezing in the open-field), and were shown to perform better in a maze (Bernstein, 1952). They also had a less pronounced response to stress suggesting that gentling had increased the threshold of their stress response (Weininger, 1956, 1954).

Interestingly, Seymure Levine put forward an alternative hypothesis to explain these findings. He suggested that handling represents for the rodent a fearful situation (the first time a rat is handled it tries to get away frantically and responds with excessive defecation) and that it is this exposure to stress early in life that prepared the rodent to respond adaptively to stress in adulthood (Seymour Levine, 1956). Indeed, Levine's findings supported the latter theory showing that handling alone was sufficient to replicate the same effects Weininger obtained with gentling. He also introduced the idea that the earlier the exposure to the handling (pre- compared to post-weaning), the more profound its effects. Apart from small variations across studies, the handling procedure generally involved picking up and moving the rat pups from the home cage with the dam to a different container for 15 minutes before returning them to the dam[4]. The procedure was repeated daily either from post-natal day (pnd) 1 to 20 (time of weaning) and referred to as 'early handling', or for 20 days after weaning and referred to as 'late handling'. When

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[4] Handling does not represent an abnormal period of maternal deprivation, because over the course of the day mothers are regularly away from the nest and their pups for periods of 20-30 min (Jans & Woodside, 1990; Rosenblatt, 1994). At the same time, the artificial and nonspecific nature of the handling paradigm is unsettling.

tested in adulthood, handled rats compared to non-handled ones weighed more, had reduced emotional reactivity, responded to chronic stress with lower secretion of ACTH and corticosterone (main glucocorticoid in rodents), had higher survival rates during prolonged food and water deprivation, and performed better at an avoidance learning task (Ader & Grota, 1969; Denenberg & Karas, 1959, 1960, 1961; Hess et al.,1969; Levine, 1957, 1962; Levine, et al., 1967; Levine & Otis, 1958; Zarrow et al., 1972). Most interestingly when looking at the differences between early- and late-handled rats it emerged that the early-handled group consistently performed better (e.g. Levine & Otis, 1958). This difference introduced the idea that there is a *critical period* for handling, outside of which the same manipulation only generates dampened effects or, in some cases, no effects at all.

It was later found that the effects on the stress response are mediated by an increased concentration of receptors for glucocorticoids in the hippocampus, a critical region in the negative-feedback inhibition of adrenocortical activity[5] (Meaney et al, 1989, 1985; Meaney & Aitken, 1985). Increased receptor concentrations lead to greater hippocampal sensitivity to glucocorticoids and enhances the negative-feedback efficacy in the handled rats. In line with behavioural findings, handling increased the glucocorticoid receptor concentration only when performed early in development (during the first postnatal week: Meaney & Aitken 1985). Handling the animal during the second postnatal week led to reduced effects and no effects were measured in animals handled during the third postnatal week (Meaney & Aitken 1985) indicating a tight critical period. The effects of handling on the stress response have been

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[5] Stressors activate the hypothalamic-pituitary-adrenal (HPA) axis. In response to a stressor the posterior hypothalamus releases corticotropin-releasing factor (CRF) which, through connections with the anterior pituitary gland triggers the release of adrenocorticotropin hormone (ACTH). ACTH, in turn, causes the release of glucocorticoids (cortisol in humans, corticosterone in rodents) from the adrenal gland. Glucocorticoids act at a number of neural sites to exert an inhibitory, negative-feedback effect over the synthesis of hypothalamic releasing-factors for ACTH. The handling effect on feedback sensitivity is mediated by an increase in glucocorticoid receptor (GR) expression in the hippocampus, a region that has been strongly implicated in glucocorticoid negative-feedback regulation. The increased hippocampal GR gene expression is therefore a central feature of the handling effect on HPA responsivity to stress, resulting in increased feedback inhibition CRF synthesis and reduced pituitary ACTH release during stress (Meaney, 2001).

measured as late as 24 to 26 months of age (Meaney et al.,1988, 1992) pointing to the long-term effects of this early life intervention.

1.1.2.2 From handling to licking and grooming

Faced with these findings on handling, different researchers started questioning whether these were not actually triggered by small changes in the mother-pup interaction that occurred as a consequence of the handling (Barnett & Burn, 1967; Bell & Smotherman, 1980). For example, Levine proposed that handling (which remains a stressful event) altered the behaviour of the mother towards the pups upon their return to the cage (Levine, 1975). Thus, it was the difference in maternal behaviour across the handled and the non-handled litters, rather than the handling intervention itself, that mediated the effects on the endocrine and behavioural response to stress. A series of studies followed that involved the observation of maternal behaviour after the pups were placed back into the cage (Bell et al., 1971; Lee & Williams, 1974; Priestnall, 1973). Bell and colleagues (1971) reported that handled rats emitted more frequent, longer and higher pitched vocalizations compared to non-handled rats. Concurrent with vocalizations, mothers where making physical contact with the pups by retrieving and grooming them, and no interactions were observed when no vocalizations were detected. Similarly, Lee and Williams (1974) observed that mothers of handled pups lick them more, but differences in amount of licking were limited to the first hour post-handling (Priestnall, 1973).

These findings confirm the belief (Levine, 1975; Bell & Smotherman, 1980) that handling modifies mothering, and suggest that these changes are mediated by vocalizations of the infant pup. The quantification of the maternal behaviours across handled and non-handled litters revealed that differences are confined to a very specific behaviour: licking and grooming (LG) (Dong Liu et al., 1997). Indeed, mothers of handled pups were found to nurse in an arched back position and to lick and groom their pups more frequently than mothers of non-handled pups, who instead assumed a passive posture during nursing and engaged less in active tactile stimulation. In their seminal work, Liu and collaborators followed these finding by measuring the naturally occurring individual differences in LG behaviour. Not only did they find that pronounced and stable individual differences existed for this behaviour, but that these could

predict the magnitude of their offspring's stress response. As adults, the offspring of high-LG dams had reduced plasma ACTH and corticosterone concentrations in response to stress as compared to the offspring of low-LG dams (Liu et al., 1997). Furthermore, the offspring of high-LG dams had a higher concentration of hippocampal glucocorticoid receptors (Liu et al., 1997).

These fascinating studies revealed that variations in frequency of maternal-LG, experienced early in life by mice and rat pups, are linked to long-lasting effects on the hypothalamic-pituitary-adrenal (HPA) response to stress (for a review, see Meaney, 2001). Most importantly these studies identified a specific time window during which this behaviour has to take place in order for such effects to be observed. Indeed, lifelong differences in rats that had high- or low-LG mothers are only ascribable to differences in mothering styles during the first week post-partum (Champagne, Francis, Mar, & Meaney, 2003).

Work in rodents reviewed thus far showed not only that early social tactile contact impacts HPA mediated responses to stress, but most importantly that it is a very specific form of tactile stimulation (licking and grooming) that produces this effect. Was this specificity conserved throughout evolution? Even though the repertoire of tactile behaviours that an infant is exposed to is much richer compared to that of a rodent pup, is it possible to pinpoint a tactile interaction that is responsible for promoting appropriate stress responses also in humans? These studies provide solid ground for asking such questions and encourage us to look more closely at social touch in our own species.

1.1.2.3 Maternal separation in rodents

Handling which consists of briefly (usually up to 20 minutes) separating the pup from the dam, does not represent an abnormal period of maternal separation (MS) for rodents. Indeed, over the course of the day mothers are regularly away from the nest and their pups for periods of 20-30 min (Jans and Woodside, 1990; Rosenblatt, 1994). To test the effects that the loss of maternal care for longer periods of time has on the pup, researchers extended the period of separation to 2 to 3 hours. Different groups measured in great detail both the short- and the long-term effects of MS. I am going to present the research that used MS (not always intentionally) to assess the impact that prolonged lack of maternal touch has on the development of the organism. If the studies on

handling led us to discover that LG modulates stress responsivity, those on MS show how lack of this stimulation for extended periods of time impacts growth. It is also through use of this paradigm that the relative contribution of passive body contact on development was discovered (see 1.1.4).

1.1.2.3.1 Effects on growth

Licking and grooming was found not only to affect the behavioural and neuroendocrine responses to stress, but also to have profound effects on physical growth. In the context of studies on brain development and the molecular mechanisms responsible for maturation and cell growth, the group led by Saul Schanberg reported having difficulties in measuring the activity of the enzyme ornithine decarboxylase (ODC) (index of cell differentiation and replication). They later discovered that maternal separation to which the rat pups in the study had been unintentionally exposed was the cause of this effect. Indeed, MS triggers the pup to enter a 'survival mode' in which the organism conserves energy until the mother returns. This early observation led to a series of rigorous studies investigating the effects of MS on the organism of the young pup (Butler & Schanberg 1977; Evoniuk et al. 1979; Pauk et al. 1986, Schanberg & Kuhn, 1980, Kuhn et al., 1978)). The effects of MS are evident as early as 20 minutes following separation and include decreases in ODC activity and in growth hormone (GH) [6] levels and an increased corticosterone secretion (for an overview of the findings see (S. Schanberg, 1995)). Reunion with the biological mother (or an accepting lactating female) reversed to baseline levels all the physiological changes induced by separation. Additionally, these effects were only observed in pre-weaning rats and not in older ones. Elegant and well controlled experiments followed that aimed at revealing which component(s) of the mother-pup interaction mediated the effects of growth. Maternal body temperature and the familiar environment (Butler & Schanberg, 1977), feeding (Butler, Suskind, & Schanberg, 1978), olfactory, visual and auditory stimulation (S. M. Schanberg & Kuhn, 1980) were all individually removed from the pup's experience, but none was found to impact growth indexes. Eventually, the

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[6] OCD activity is usually measured in one or more tissues including brain, heart and liver. Growth hormone is secreted from the anterior pituitary gland.

group showed that growth impairment was associated with the lack of active tactile stimulation (licking and grooming) obtained via anesthetization of the mother (Butler, Suskind & Schanberg 1978). Thus, denying the pup access to this specific behaviour led to severe growth impairments, even in the presence of many other passively transferred sensory cues[7]. In support of this finding, exposing the mother-separated pup to tactile stimulation that resembled the tongue licking behaviour of the mother (using a wet paintbrush), reversed the effects of MS (Evoniuk et al., 1979; Pauk et al., 1986).

Furthermore, in order to assess that growth was regulated by licking and grooming specifically and not by the stimulation of other systems that could co-occur during the vigorous stroking pattern applied with the brush, different forms of stimulations were tested. The comparison of the differential effects of vestibular (rocking), kinaesthetic (passive movement of the limbs) and tactile (brush stroking) stimulation on both growth (OCD activity and GH levels) and the stress response (corticosterone levels) (Pauk et al., 1986) revealed that only stroking reversed the effects of MS.

1.1.2.3.2 Effects on stress responsivity

Some of the aforementioned studies (par. 1.1.2.3.1) measured the immediate impact that MS has on glucocorticoids levels, and showed that maternal tactile stimulation dampens HPA activity in neonates, possibly protecting the animals against the highly catabolic effects of adrenal glucocorticoids during a period of rapid development (see Levine, 1994). However, the question these studies did not address is that concerning the long-term effects of MS on stress responsivity. How does MS, compared to postnatal handling, affect HPA mediated stress responses? This question was investigated in detail in a group of studies by Michael Meaney's group (e.g. Huot, Plotsky, Lenox, & McNamara, 2002; Ladd et al., 2000; Liu, Caldji, Sharma, Plotskyt, & Meaney, 2000; Plotsky & Meaney, 1993; Plotsky et al., 2005). Animals that for the first 14 days of life were separated from the mother for 3 hours once a day were then tested as

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[7] In this experiment pups were in the cage with their mother (anesthetised) and littermates, so they were exposed to sensory cues transmitted passively from the mother and actively from the littermates. The fact that active tactile stimulation from the littermates does not modulate OCD activity highlights the specificity of LG.

adults in a stress inducing situation.   Findings from this body of work revealed that the effects of maternal separation on stress reactivity are exactly the opposite of those associated with handling and exposure to high levels of naturally occurring LG: increased HPA responses to acute stress, decreased levels of hippocampal glucocorticoids and blunted feedback sensitivity.

These results suggest that early life stress in the form of maternal care deprivation (LG stimulation in particular) for 3 hours a day leads to heightened stress responsivity later in life. LG stimulation after MS cannot compensate for its prolonged absence and prevent the long-term effects. Similarly to handling manipulations, dams lick and groom their pups more after MS compared to the non-handling condition. The difference in LG following handling and MS is not clear, as some studies reported that pups receive relatively less LG after MS compared to handling (Boccia & Pedersen, 2001; Francis & Kuhar, 2008) while others instead found that MS led to increased LG compared to handling (Biggio et al., 2014; Pryce, Bettschen, Nanz-Bahr, & Feldon, 2003; Zimmerberg & Sageser, 2011, but see Lundberg, et al., 2017 for finding a difference in levels of nursing and not LG). Despite inconsistency in these findings, evidence clearly shows that as a consequence of MS, maternal care is increased, however its effects are limited. Indeed, adjusting the level of care to compensate for the long period of maternal absence is sufficient to immediately decrease baseline glucocorticoids levels but not to prevent the long-term effects on stress responsivity. Levels of LG after MS seem, however, to be a protective factor for later substance abuse: the more LG animals receive as pups, the lower the amounts of alcohol and cocaine they self-administer as adults (Francis and Kuhar, 2008).

Taken together the findings from MS studies build on those from handling suggesting that LG has immediate impact on endocrine function and that prolonged lack of LG has long-term non-reversible effects on stress responsivity. Additionally, these findings expand on those from the handling literature pinpointing the direct link between LG and release of growth hormone in the postnatal period.

Revealing the mechanisms through which active tactile stimulation modulates the stress response (HPA axis) and growth (ODC and GH) opens the possibility of translating this research to humans. Has early tactile contact

conserved the same regulatory function on HPA activity and on growth? Measuring long-term effects in humans poses methodological challenges, as we would have very limited control on the variables that differentiate the experimental and the control groups between time of stimulation and assessment of the effects later in life. Instead, the short-term effects of early tactile stimulation can be more easily tested in human infants.

1.1.2.4 Further considerations on licking and grooming

Following the discovery that LG exerts a pervasive influence on development, this behaviour was closely investigated. Observations of maternal behaviour showed that mothers differ between each other in the amount of LG only over the first 6– 8 days postpartum and no differences are measured after the first postnatal week. This finding supports and offers an explanation for the critical period for LG. Frequency data indicate that the frequency of LG is normally distributed across dams (see Figure 1.2) and that individual differences in LG are rather stable as these can be observed across multiple litters (Champagne et al., 2003). Furthermore, these differences are transmitted from one generation to the next. As adults, the female offspring of high LG mothers show significantly more LG compared with the female offspring of low LG mothers (Francis et al., 1999). This transmission is nongenomic as it was shown by cross-fostering studies (Francis et al 1999), and epigenetic mechanisms have been discovered (see Champagne, 2008 for a review).

In addition to the effects on growth and on HPA activity, differences in levels of LG early in life have also been shown to have long-term impact on certain aspects of cognition, such as memory and learning, and on vulnerability to drug use (e.g. Bredy et al., 2003; Francis & Kuhar, 2008; Liu et al., 1997; Zaharia et al., 1996).
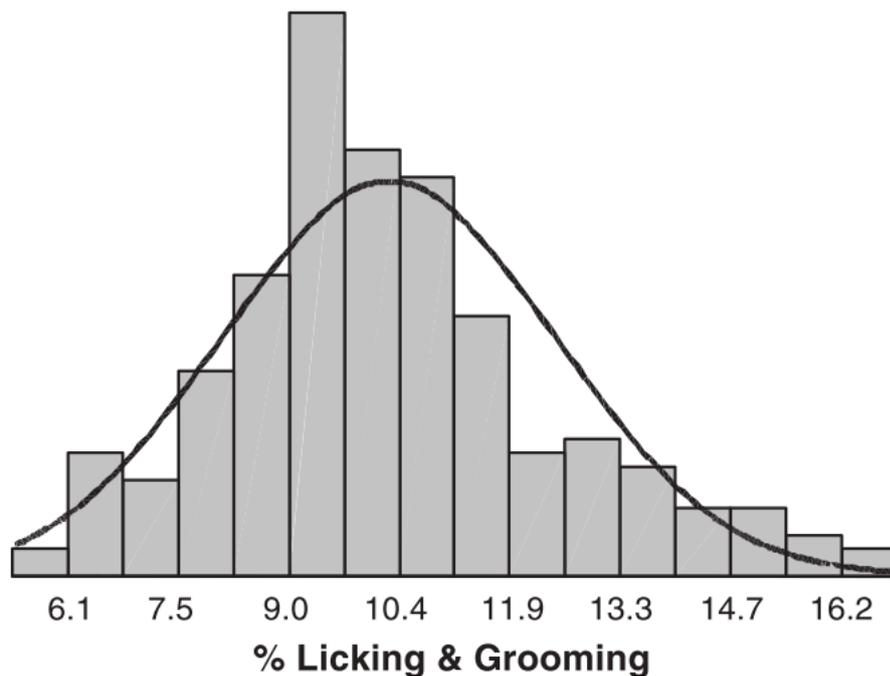
Figure 1.2 Frequency distribution of cumulative licking/grooming during the first 6 days postpartum. Superimposed is a computer-generated normal distribution. From Champagne et al., 2003

Research presented thus far would suggest that being raised by a high-LG (vs. low LG) dam, and by the same token being handled, is beneficial for the organism as it results in lower levels of stress-induced glucocorticoids. In rodents, glucocorticoids levels have been associated with hippocampal degeneration and the emergence of learning and memory deficits (Issa et al., 1990; Landfield et al., 1981; Landfield & Pitler, 1984; Sapolsky et al., 1984). However, an advantage has been shown for non-handled animals as well (exposed to lower levels of LG compared to handled ones), since their higher levels of glucocorticoids protected them from the rodent model of Multiple Sclerosis (Laban et al., 1995). Thus, we cannot conclude that handled animals are better adapted than non-handled ones: they are different. Supporting this idea, the variability of LG suggests that the two phenotypes (high- and low-LG) could be equally adaptive. To understand the adaptive advantage of having increased stress reactivity (besides being protected from certain diseases) we need to look at the type of (naturalistic) environment in which rodents grow up. For example, being raised in highly adverse conditions implies a less present

mother (low LG), since most of her energetic expenditure will be invested in facing the demanding environment. In this scenario, dams raise animals with an enhanced level of stress reactivity as if to prepare them for the high level of environmental adversity. On the other hand, a safer environment will allow the mother to spend more time taking care of her pups, signalling to them the quality of their future surroundings.   The hypothesis that the maternal environment influences the level of maternal care was tested by exposing to stress pregnant dams that were previously defined as high-LG (Champagne & Meaney, 2000). In support of the hypothesis, these dams showed low levels of LG behaviour with the offspring. Therefore, both phenotypes have adaptive advantages as they seem to tune the pups' physiology and behaviour for the expected environment.

1.1.4 The construct of 'maternal proximity'

Myron Hofer, motivated like Harlow by an interest in attachment, once observed the pervasive effects that maternal separation had on a rat that had escaped the cage overnight (the same 'survival mode' that Schanberg had described in his studies). He designed a series of elegant experiments to understand how different components of the mother's physical presence affect infant's physiological homeostasis. Each component was provided separately to rat pups in order to measure what specific systems in the pup it regulated (Hofer, 1994). As reported above, Schanberg's group had also run studies where the mother separated pups were provided with different aspects of the mother-pup interaction (Butler et al., 1978) (Butler & Schanberg, 1977; Butler, Suskind & Schanberg 1978; Schanberg & Kuhn, 1980). However, while Schanberg's work only measured effects on growth indexes and on glucocorticoids, Hofer's work measured the specific impact of each aspect of maternal presence (thermal, olfactory, nutrient, tactile, sensorimotor) on multiple systems (behavioural, neurochemical, metabolic, sleep-wake cycles, cardiovascular, endocrine, and immune). From this extensive work, it emerged that different aspects of maternal presence contribute specifically to the regulation of different aspects of physiologic and behavioural development. While nutrition seems to be the only regulator of the cardiovascular system, combinations of

olfactory, thermal and tactile components were found to regulate activity level, sleep-wake cyclicity and arousal, and endocrine system (Hofer, 1994a; 1994b). Touch does not regulate these systems on its own, but without touch these systems are dysregulated.

For example, separation from the dam and relocation to unfamiliar surroundings is immediately followed by pup's high intensity vocalizations (described also in the handling literature) and hyper reactivity. In order to understand what sensory stimulation could attenuate the vocalizations Hofer exposed the pup to test stimuli such as artificial fur, contoured surfaces, warmth and scents taken from the home cage nest, either individually or in combination. He found that vocalizations decreased as the number of familiar sensory modalities increased: a rubber model that was warm but odourless and lacked soft texture had no effects; a piece of soft fur alone had some effect; a warm object covered in fur and familiar odours almost entirely reduced vocalizations (M. A. Hofer & Shair, 1980). Further, vocalizations decreased as the pup's contact time with the stimuli increased. Furthermore, the agitated behaviour and increased behavioural reactivity that follows separation was attenuated by provision of tactile or maternal olfactory stimulation, suggesting that both sensory stimulations are equally important in regulating behavioural hyper responsivity (M. A. Hofer, 1975).

Hofer studied separation effects in two-weeks-old rat pups, the age of weaning in rodents. Thus, these animals are older than the animals tested in the handling and the MS experiments presented earlier, which suggests that if a critical period for maternal proximity exists, this is not as tight as the one for LG.

These studies add to our understanding of the role of touch early in life by showing that during development maternal proximity is crucial for promoting physiologic and behavioural development. While it cannot be concluded that touch is the only sense responsible for these effects, as during maternal proximity thermal and olfactory components are provided as well, there is no maternal proximity without touch.

In rodent models while active tactile stimulation (LG) specifically promotes growth and regulates the HPA mediated stress response, passive contact with the mother has a fundamental role in ensuring regulation of

physiology and behaviour in the newborn pup. On the one hand, mechanisms that mediate LG effects have been identified and can direct research with human infants (e.g. measuring cortisol). On the other hand, mechanisms behind how passive maternal contact specifically contributes to regulation across systems are not clear, therefore comparisons across species can only be drawn at the behavioural level.

Returning to the two questions that opened this thesis, rodent models, unlike non-human primate models, could answer the first question (*how does social touch promote development?*) by unveiling the mechanisms through which maternal touch promotes development. Mechanisms identified with this body of work are: regulation of the HPA axis, upregulation of the expression of glucocorticoid receptors in the hippocampus, regulation of GH and of ODC enzyme activity. As concerns the second question (*what defines social touch?*), this work showed that licking and grooming and passive body contact are both forms of social touch important for development, each exerting a different function. Are active and passive tactile stimulation crucial also for human infants? The next section will present work from preterm human infants that can serve to answer this question.

1.2 Touch in human early maternal separation models

Work in animal models reviewed thus far indicated that while touch is crucial to the development of both non-human primates and rodents, species-specific differences exist as to what type of contact is important. While for young rhesus macaques 'contact comfort' is both necessary and sufficient for (behavioural) development, for young rodents passive body contact with the dam is necessary for the regulation of multiple physiological systems but it is not sufficient for the pup's survival. Indeed, it is another type of tactile stimulation (the licking performed by the dam) that regulates growth and the HPA axis (see Table 1.1 for a summary of the effects). Interestingly, dysregulation of this tactile interaction is restricted to a tight critical period, the first postnatal week.

In light of these compelling findings, the question of whether throughout evolution the role of touch remained unaltered for early development arises. Specifically, I ask: (i) does the sense of touch prevail over the other senses in promoting development and (ii) is it a specific type of touch that is alone responsible for these effects? These questions are as interesting as they are challenging, one of the obstacles being that experimentally induced maternal separation is not a viable option in humans.

An opportunity to study the role of early tactile contact comes from the maternal separation model offered by preterm birth. Time spent in the incubator in Neonatal Intensive Care Units (NICU) for both preterm and very low birth weight (VLBW) infants can be viewed as a limbo between life in the womb, and exposure to maternal care. Indeed, for a preterm born infant a portion of prenatal development has to take place outside of the womb under conditions of (persistent) maternal separation. The longer the period of time that elapses between preterm birth and access to maternal care the more detrimental the effects on infant development. Negative impacts on arousal regulation, stress reactivity, attention and learning were shown to persist late into adolescence (Allin et al., 2001; McCormick et al., 1996; Ruff, 1986).

Given the lifelong effects of prematurity, a number of interventions have been tested with the aim of reversing or preventing part of these damages. What these interventions have in common is that they all provide supplemental sensory stimulation, the rationale being that the sensory environment to which an infant is exposed when in NICUs is far from optimal. In NICU, infants are firstly overstimulated with continuous light and noise and repeatedly exposed to painful procedures which their immature systems may not process (Als, 1991) and secondly exposed to minimal tactile and proprioceptive stimulation.

NICU interventions have been designed to mimic either the intra- or the extrauterine environment (Cornell & Gottfried, 1976). Such choice is dependent on whether researchers believed that the infant would have benefitted more from stimulation that resembled life in the womb (e.g. vestibular stimulation and exposure to heartbeat sound) or from stimulation typical of the mother-infant relationship (e.g. tactile-proprioceptive stimulation and exposure to recordings of the mother's voice) (for a review of different types of supplemental stimulation see Dieter & Emory, 1997).

It is since the 1960s that a substantial number of interventions employing supplemental tactile stimulation has been the objects of clinical studies. Different research groups measured the effects of various forms of touch (e.g. stroking, rubbing, passive extension of the limbs, rocking, waterbeds, non-nutritive sucking) and the early findings converged to support the idea that touch per se aids development. Vestibular stimulation in the form of rocking led to weight gains and improved ability to track visual and auditory stimuli (Freedman, Boverman, & Freedman, 1996; Neal, 1968). Oscillating water beds in incubators reduced the incidence of apnoea (uncomplicated forms of it) (Korner et al., 975) and facilitated sleep and decreased restlessness in preterm infants treated for more complicated forms of apnoea (Edelman et al., 1982; Korner et al., 1978, 1982). Tactile and kinaesthetic stimulation (gentle stroking or rubbing of the skin and passive movements of the limbs) was reported to increase weight by some studies (Rausch, 1981; Solkoff et al., 1969; White & Labarba, 1976) but such results were not replicated in other studies (Barnard, 1973; Freedman et al., 1996; Hasselmeyer, 1964; Kramer et al., 1975; Solkoff & Matuszak, 1975). Another inconsistent finding related to tactile kinaesthetic intervention concerns activity. The stimulation was reported to increase levels of gross motor activity following the stimulation by some (Scott et al., 1983; Solkoff and Matuszak, 1975, Solkoff et al., 1969) but not by others (Hasslemeyer, 1964; Barnard, 1973). It has been suggested that these discrepancies are due to variability in sampling parameters and use of non-standardized stimulations (Schanberg & Field, 1987). Even the tactile stimulation of a small body surface like the mouth (through non-nutritive sucking) had positive effects on weight gain and early hospital discharge (T. Field et al., 1982; Measel & Anderson, 1979).

Certainly, these early works showed that the development of touch-deprived preterm infants benefits from the implementation of a wide range of tactile contacts. However, the well-known effects of touch on infants born preterm is owed to two interventions that underwent a more systematic investigation: infant massage and mother-kangaroo-care.

1.2.1 Infant Massage

Tiffany Field and her research team produced the largest body of work on the effects of massage therapy in preterm infants. The original motivation behind her work on massage was that of exploring whether findings from Scahnberg's group on rodents applied also to preterm human neonates. Massage therapy consists of moderate pressure stroking (tactile stimulation) often accompanied also by flexion and extension of the upper and lower extremities (kinaesthetic stimulation) performed for 15 minutes, two to three times a day for a minimum of 5 days in a row. Field's studies reliably show how infants assigned to the massage group have greater weight gains (21-48%) and shorter hospital stays (3-6 days)[8] than controls. Behaviourally the massaged infants are more active and alert and perform better on the Neonatal Behavioural Assessment Scale (NBAS; Brazelton, 1973). These results have been replicated by several groups across countries and cultures (e.g. Cifra & Sancho, 2004; Ferber et al., 2002; Mathai et al., 2001) and studies on massage therapy have been the object of several reviews (Álvarez et al., 2017; Tiffany Field, 2016, 2017; Tiffany Field et al., 2011; Pepino & Mezzacappa, 2015; Vickers et al., 2004) and meta-analyses (Badr, Abdallah, & Kahale, 2015; Li, Zhong, & Tang, 2016; Wang et al., 2013).

The component of massage that seems responsible for the gain weight is the moderate pressure. Indeed, when compared with infants who received light pressure stimulation, the moderate pressure massage group gained more weight and showed decreased heart rate and higher vagal activity[9] (Field et al., 2006). A mechanism was suggested that stimulating pressure receptors, as with massage, (baroceptors and Pacinian corpuscles in the skin) increases vagal activity, and vagal stimulation facilitates gastric motility and the release of insulin, and indirectly leads to the release of insulin-like-growth-factor-1 (IGF-1), which plays a key role in regulating preterm infant growth (Field et al., 2011a).

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[8] Weight gain is one of the main criteria for discharge from the Neonatal Intensive Care Unit (NICU).

[9] The vagus nerve is the prime component of the parasympathetic nervous system. It innervates most organs in the body including the gastrointestinal and cardiovascular systems. The measure of heart rate variability offers an indirect measure of vagal activity.

The parallels between these findings and those on rodents are striking. In line with findings from animal models, active tactile stimulation was found to promote growth in human neonates. While the mechanisms behind this effect have not been entirely understood yet, both in rat pups and in human infants growth is not mediated by caloric intake. Additionally, comparing the differential effects of kinaesthetic and tactile stimulations, Field's group showed that, as in rodents, only the tactile component is responsible for the effects on gain weight (Schanberg and Field 1987). This research suggests that in human (preterm) infants growth is facilitated by a specific type of tactile interaction, one that resembles the licking action of the rat dam.

Effects of massage on cortisol, the primary glucocorticoid in humans which corresponds to corticosterone in rodents, were measured by a handful of studies which yielded inconsistent results. Baseline cortisol was shown to decrease in some studies (Acolet et al., 1993; Schanberg et al., 1996) but not in others (Kuhn et al., 1991; Field et al., 1990) and a high variability of responses was reported in another work (Gitau et al., 2002). Therefore, compared to rodent models, in human preterm infants active tactile stimulation does not seem to be sufficient on its own to regulate the HPA axis in the short-term. Whether massage therapy affects stress responsivity of infants born preterm later in life has not been assessed and, in general, studies that followed cohorts of massaged vs. non-massaged infants longitudinally are scarce (e.g. Ferber et al., 2005; Procianoy, Mendes, & Silveira, 2010).

While the impact on weight gain and, as a consequence, on the length of hospitalization, are the findings that emerge most consistently from reviews and meta-analyses, other measures seem to benefit from the massage therapy. For example, massage was linked to an increase in immune system activity (i.e. activity of natural killer cells) (Ang et al., 2012); pain reduction during invasive procedure (Abdallah et al., 2013); better motor development scores (Fucile & Gisel, 2010); better mental development at a two-year follow-up assessment (Procianoy et al., 2010); better mother-infant relationship at 3 months (Ferber et al., 2005). While compelling, the finding that the effects of massage can be measured months later after its administration in a number of domains (including the cognitive one) still needs to be systematically replicated. Another

type of supplemental tactile stimulation underwent more rigorous investigation of both short- and long-term effects on development: Kangaroo Mother Care.

1.2.2 Kangaroo Mother Care

Kangaroo Mother Care is a technique that was devised out of necessity. In 1978, faced with a shortage of incubators, in Bogota, Colombia, doctors turned to mothers as natural incubators and instructed them to place the naked infants between their breasts. The 'Kangaroo position' served a double purpose: to regulate the infant's body temperature through maternal body heat and provide nutrition through exclusive breastfeeding. A series of randomized clinical trials showed that this intervention was safe and it did not increase mortality rates as compared to standard incubator care (Charpak et al., 1997; Sloan et al., 1994; Whitelaw & Sleath, 1985). Most interestingly, besides safety of use, early pilot studies run in Bogota's hospitals revealed that KMC led to better outcomes across several dimensions compared to incubator-based care. A pattern of unexpected positive effects associated to KMC started emerging, ranging from greater weight gain (Kambarami et al., 1998) to the stabilization of the infant's physiology (Fischer et al., 1998; Ludington & Golant, 1993), to better thermoregulation and oxygenation (Acolet et al., 1989; Bauer et al., 1996; Bier et al., 1996; Bosque et al., 1995; Ludington-Hoe & Swinth, 1996; Törnhage et al., 1998).

In the early 1990's this method had spread to industrialized countries where parents of preterm infants were offered the option to spend a portion of the day in the kangaroo position (while the infant was still attached to monitor devices). As more countries adopted KMC, evidence of its beneficial effects started accumulating. Some findings suggested a role of KMC in attenuating the stress response, as it was found to reduce crying (Michelsson et al., 1996), beta-endorphins levels (Mooncey et al., 1997) heart rate and behavioural pain scores (Mörelius et al., 2005). KMC was found to reduce the increase in heart rate and the amount of crying following a painful procedure (Ludington-Hoe et al., 2005). Another positive effect often reported was on state regulation, increasing both quiet sleep and alert wakefulness (Gale, Franck, & Lund, 1993; Gale & VandenBerg, 1998). However, directly assessing the impact of skin-to-

skin contact on baseline levels of the stress hormone cortisol led to inconsistent findings as one group reported no changes (Mooncey et al., 1997), another group showed a significant decrease (Gitau et al., 2002) and a third group showed changes in both directions in their sample (Morelius et al., 2005). A review was published marking 25 years since the introduction of KMC intervention and it reported widely replicated findings on improvements in the following measures: weight gain, body temperature, breastfeeding, behaviour (the kangaroo position has a calming effect on babies who typically fall asleep), behavioural indexes of stress, cognitive development and mother-infant bonding (Charpak et al., 2005)

1.2.2.1 A consideration on the short-term effects of massage and KMC on gain weight and cortisol

Since both active (massage) and passive (KMC) tactile contact have been reported to have an immediate effect on gain weight, it suggests that in human infants, as compared to rodents, a broader range of social touches[10] can regulate growth. This makes sense if we consider that non-human primates still thrive even if raised by surrogates that cannot provide any active stimulation. Perhaps during evolution maternal proximity became sufficient to signal an environment in which it was safe to thrive.

As far as the effects on cortisol are concerned, both interventions seem to lead to inconsistent findings. While the short-term effects on weight gain are evident and widely replicated, suggesting that the function of social touch on growth is conserved (despite the fact that the mechanisms have not been revealed yet), the effects on cortisol seem far more elusive. Although neonatal units represent a stressful environment for the preterm infant, probably its effect on cortisol levels are not as pronounced as in mother deprived rodents. A possibility is that the impact on cortisol is modulated by the number of painful

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[10] I refer to 'social touch' earlier in this paragraph because in these studies we cannot isolate the effects of touch per se from those linked to being touched by a conspecific, and no studies compared human generated touch to touch delivered through an inanimate object (e.g. a brush). Therefore, when discussing the importance of touch for development we refer to social touch defined as any touch occurring between conspecifics.

procedures an infant is exposed to and by levels of stimulation experienced in different NICUs. Indeed, the level of attention in shielding the infants from light and sounds could vary both within and across units, confounding the results. The inconsistency between studies might be enhanced by the fact that very few studies measured baseline cortisol after tactile interventions.

1.2.2.1 The Israel Kangaroo Care project

Alongside studies that measured short-term effects of KMC during the hospitalization period, different groups started follow-up studies of treated versus non-treated infants to investigate the long-term effect of the intervention. The most comprehensive longitudinal study on the effects of KMC was carried out by Professor Ruth Feldman and her research group and is known as the "Israel Kangaroo Care project". 146 low birth-weight preterm infants and their families were enrolled in the study: 73 of them received the KMC intervention for at least one hour a day for at least 14 consecutive days and 73 case matched infants served as controls. In the experimental group, the KMC intervention was always provided by mothers. Infants and their families were assessed at eight time points: before the intervention, at term age, at 3, 6, 12 months and at 2, 5 and 10 years. Results are striking and overall show the multidimensional impact that this intervention has across development. Positive effects have been measured on infant self-regulation and neuromaturation, on maternal well-being and mood and on the mother infant and family relationships.

As for self-regulation, infants who received KMC in the neonatal period had a more organised *sleep-wake cycle* and spent more time in quiet sleep and alert wakefulness, confirming findings from previous research (Gale et al., 1993; Gale and Vandenberg, 1998). Spending time in these two states indexes optimal state organization and is of paramount importance as it affords the infant rest (quiet sleep) and active exploration and information intake (alert state). Preterm infants usually spend longer periods in transitory and unfocused states and have disrupted sleep-wake cyclicity. At 3 months, arousal modulation and emotion regulation were assessed measuring the infants' response to a series of increasingly intrusive stimuli (from simple unimodal to complex multimodal ones). Infants in the KMC group had better emotion regulation with higher

threshold to negative emotionality, indexed by longer latencies to the first cry. They also had better arousal modulation since they could flexibly switch between medium levels of arousal during stimulus presentation (optimal for information processing) and lower levels of arousal when the stimulus presentation ended (Feldman et al., 2002). At 6 months, in the context of a mother-infant exploration session, infants in the intervention group showed more sustained exploration and had longer periods of joint attention (Feldman et al., 2002).

Regulatory functions were shown to discriminate the two groups beyond the first year of life. Children who had received KMC showed *less distress* during maternal separation at 1 year, and better *executive functions* at 2 and 5 years (Ruth Feldman, 2011). The intervention group continued to show improved executive functions also at the 10 years' time-point, compared to controls. Furthermore, *sleep organization*, another measure collected at this time-point, was better for the KMC group, with controls scoring overall as poorer sleepers (Feldman et al., 2014).The effects of skin-to-skin contact on self-regulation are long-lasting and differences between the treated and the non-treated groups can still be captured 10-years post intervention.

As far as KMC impacts the infant's development (as opposed to the mother or the mother-infant relationship) effects on neuromaturation were also measured. One observation of maturation was vagal tone. This is the measure of how respiration impacts heart rate variability as mediated by the parasympathetic system and is an index for the maturity of the nervous system. In preterm infants, immaturity of the neurological systems leads to lower vagal tone, which does not reach maturity at term age. Feldman and colleagues found that while at term age vagal tone had reached maturity in both groups, infants who had received KMC had a higher vagal tone compared to controls, to indicate that a quicker neuromaturation had taken place in this group (Feldman & Eidelman, 2003). When measured at 10 years, vagal tone was still higher in the intervention group (Feldman et al., 2014). In addition, neuro-developmental maturation was assessed with the NBAS. At term age infants who received KMC had higher scores on both the habituation and the orientation clusters of the scale, indexing more efficient information processing (Feldman & Eidelman 2003).

Alongside regulatory functions and neuromaturation, cognitive development was also positively impacted by the intervention. Indeed, KMC was shown to promote cognitive development (general IQ) during the first two years of life, but no differences between groups are measured at either 5 or 10 years[11]. Instead, at these two time points, the KMC group shows better outcome on executive functioning tests suggesting that as the infants grow, early maternal contact becomes less associated with general intelligence and more with regulatory skills (Feldman et al., 2014).

More importantly, just as in rodent models tactile contact had long-term effects on stress responsivity: 10 year old children who as infants received KMC, show lower cortisol reactivity compared to controls (measured with the Trier Social Stress Test for Children; children make public speech and compute complex arithmetic before unfamiliar judges) (Feldman et al., 2014). The impact of skin-to-skin contact on stress reactivity is further supported by a recent study that reported milder cortisol reactivity in 1-month-old infants who had received continuous skin-to-skin contact while in NICUs (Mörelius et al., 2015).

Overall, implementing maternal contact in the form of skin-to-skin contact during the first two weeks of life showed long-lasting effects across the first ten years of life. Beyond the neonatal period, a number of measures including cognitive development, executive functioning, autonomic functioning, sleep organization and the stress response benefitted from the provision of early contact. While the mechanisms mediating such effects have not been tackled it is possible that these are a consequence both of the early organization of the biological clock and of regulation of the HPA axis. On the one hand, organised sleep-wake cyclicity could be the foundation for regulation of the arousal system, emotion regulation and attention, in line with existing theories (Dahl, 1996; Sander, 1983; Wright & Harding, 1992). On the other hand, regulation of the HPA axis (possibly via increased hippocampal glucocorticoid receptors?) could mediate the effects on stress reactivity and also those on executive functioning. Indeed, chronic activation of this axis has been shown to affect the development of neural systems, including the hippocampus and the

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[11] At 6, 12 and 24 months IQ was tested with the Bayley Scale of Infant Development; at 5 years with the Wechsler Preschool and Primary Scale of Intelligence; at 10 years with the Wechsler Intelligence Scale for Children.

prefrontal cortex, important for executive functions (in non-human primates: Bachevalier et al., 1997; Collins et al., 1998; and in rodents: Floresco et al., 1997; Goldstein et al., 1996).

Kangaroo Mother Care was not an intervention designed to answer a specific research question. However, it fitted well with what we knew from animal research. In line with Hofer's findings, it made perfect sense that maternal proximity in the immediate post-birth period promoted regulation of different systems (e.g. sleep-wake cyclicity, arousal states) and that preclusion thereof led instead to dysregulation.

I opened this section asking whether in human infants (i) the sense of touch prevails over other senses in promoting development and (ii) whether a specific type of touch is alone responsible for these effects. Studies of both infant massage and KMC might tempt us to answer the first question in an affirmative way. However, the pervasiveness of the effects of touch might be explained by the fact that at the earliest stages of development there is a critical period for somatosensory stimulation. These findings could actually lend support to Gottlieb's theory of the ontogeny of sensory development (Gottlieb, 1971) according to which sensory development occurs in a sequential order and the primary senses of touch and proprioception precede the secondary senses of vision and audition. Thus, providing tactile stimulation during periods when the sequential development of the senses is disturbed (as in a loud and bright NICU) contributes to the organization of behaviour. However, works presented thus far only compared tactile stimulation (massage or skin-to-skin contact) with no stimulation but never with stimulation in other sensory modalities (i.e. sound or vision). Therefore, touch is certainly of importance but its relative contribution compared to other senses cannot be inferred at this point.

To answer the second question, these studies indicate that development benefits from two different forms of social tactile stimulation, a static (skin-to-skin contact) and an active one (massage). These findings show a continuity with findings in rodent models but also obvious differences. In line with work in rodents providing the mother-deprived infant with passive body contact is shown to regulate the sleep-wake cycle and arousal states. Also in line with animal models, active tactile stimulation promotes growth (weight gain).

However, growth in preterm human infants benefits also from passive body contact suggesting that perhaps not one specific touch but a subgroup of social touches can afford this effect in our species. An early work (Schanberg and Field, 1987) showed that passive movement of the limbs in preterms has no impact on weight gain, so passive full body contact and massage must be processed by the developing infant as different from kinaesthetic stimulation. In terms of stress reactivity, since the effects of massage on cortisol reactivity were not tested, we cannot conclude that a passive but not active tactile stimulation modulates these.

Thus, it remains unknown whether certain types of social touches promote development, in humans. In addition, given most research was carried out with pre-term infants, the question to be asked is whether early tactile stimulation has any effects on the development of infants born at term.

Species	type of touch	effect on development	duration	nature of stimulation	mechanisms
Rhesus macaques	contact comfort	• attachment promotion • physiological stress • emotional reactivity and stress in open-field	short/long term	touch (terrycloth)	?
Rodents (mice and rats)	active tactile stimulation (LG)	growth	short	touch (brush)	ODC, GH
	active tactile stimulation (LG)	baseline HPA activity	short	touch (brush)	HPA
	active tactile stimulation (LG)	HPA responsivity	long	mother	Hippocampal glucocorticoid receptors
	passive body contact	• sleep-wake cycle • arousal states • activity levels • endocrine system	short	mother	?
Humans	active tactile stimulation (massage)	growth (weight gain)	short	mother	
	passive body contact (KMC)	growth (weight gain)	short	mother	
	passive body contact (KMC)	HPA responsivity	long	mother	
	passive body contact (KMC)	• sleep-wake cycle • arousal states • neuromaturation (indexed by *vagal tone* and *NBAS* scores on habituation and orientation scales) • general IQ • executive functioning	short/long	mother	

Table 1.1 Table showing types of touch used with animal models (rhesus macaques and rodents) and human infants born preterm.

1.3 Touch in typically developing infants

The previous research with pre-term infants has been readily extrapolated to typical development. This extrapolation seems to be also motivated by the fact that work using animal models was carried out in pups born at term. However, while rodents remain highly dependent on touch for a period after birth, human

hearing and vision develop rapidly. With these senses available to them, term born human infants may therefore not need to rely as critically on touch.

There are reasons to suggest this is not the case. Touch is the first sensory modality to develop in the human embryo. Histological evidence showed that cutaneous and trigeminal somatosensory receptors mature between the 4th and the 7th gestational week (Humprey, 1964). Prenatally, the functional development of touch also precedes that of other senses. According to behavioural evidence responses to touch have been observed since the 6th gestational week onwards (the fetus will move if its lips are touched), with grasp and rooting reflexes observed as early as the 12th gestational week (Hooker, 1943, 1952, Humprey, 1964; Moon and Fifer, 2011). For a complete account of the emergence of anatomy and function of the different sensory systems during gestation see Bremner et al., 2012 and Gottlieb, 1971.

At birth (as well as for the rest of life) the skin is the largest of our sensory organs. After birth, touch offers the very first means of contact with the world and touch is abundant in the experience of a newborn infant. Indeed, since humans are born critically underdeveloped compared to other mammals and their developmental rate is also much slower, the parental care they need in order to thrive is of a higher level and more prolonged compared to that of other young mammals. Touch mediates most episodes of parental care (carrying, feeding, changing, soothing) throughout development and especially until a child is able to walk independently. Imagining an interaction between a mother and her infant without touch is impossible and such interaction, if it happened, would be unnatural.

Given the importance of touch (as the first sense to develop and the most developed one at birth) and its abundance (the prolonged development of human infants compared to other mammals affords them higher levels of parental care almost entirely mediated by touch) it is striking that little research has addressed how infants process touch and what impact social touch has on development.

A great proportion of existing work was aimed at quantifying the repertoire of touches caregivers (mostly mothers) use when interacting with their infants and measuring their effects on the infant' behaviour. These studies inferred that social touch is used for a number of purposes, including infant's

regulation, soothing and communication. Alongside this line of research, which is of paramount value, studies investigating the mechanisms behind the positive effects of touch in humans were rare at the outset of this PhD and are only now starting to become more prevalent.

I will now briefly review current knowledge on how touch impacts early development. I will first review studies observing the use of touch in social interaction and its effects on behaviour and then discuss potential underlying mechanisms, by reviewing studies investigating the physiological effects of touch.

1.3.1 Behavioural studies on touch

Starting in the 1980's and 90's a large body of work addressed systematically the question regarding the function(s) of touch in infancy through the lens of behavioural studies. Episodes of spontaneous, face to face interactions between a mother and her infant have been an essential tool to study the development of different aspects of social relationships (e.g. Kaye & Fogel, 1980; Field et al., 1986). However, while facial and vocal behaviours have been extensively analysed tactile behaviours have often been omitted.

A close investigation of how touch mediates mother-infant interactions revealed that maternal touch subserves different purposes. For example it emerged that touch is used to attract, maintain and recapture infants' attention (Gusella et al., 1988; Jean & Stack, 2009; Kaye & Fogel, 1980; Roggman & Woodson, 1989; Symons & Moran, 1987), to soothe infants following stress or distress (Jean & Stack, 2009; Moreno et al., 2006; Stack & Muir, 1990; Weiss et al., 2000)to promote emotion regulation (Hertenstein & Campos, 2001; Weiss et al., 2000), and regulate infants' affect (Peláez-Nogueras et al., 1996, 1997, Stack & Muir, 1992, 1990b). Additionally, infants seem to prefer interactions that include touch episodes (Brossard & Dècarie, 1968). Specifically, in a group of 3- to 5-month-old infants active forms of touch (e.g. being picked up) reinforced infants smiling more effectively than static touches (Brossard & Dècarie, 1968). A social stimulation that includes touch promoted positive affect (smiling and vocalizing) and reinforced eye contact behaviours in 1-3-month-old infants more than social stimulation in the visual and auditory domain only (Pelaez-

Nogueras et al., 1996). Pelaez-Nogueras and colleagues subsequently compared the reinforcement effects of tickling/poking to stroking on 2- to 4.5-month-old infants when they made eye contact with the experimenter. Infants in the stroking condition smiled and vocalized more and cried less than infants in the tickle/poke condition (Pelaez-Nogueras et al., 1997).

Another tool that proved to be precious in revealing the different functions of touch is the still-face (SF) paradigm (Tronick et al., 1978). This is a modified version of the typical mother-infant interaction and consists of three brief periods (90-120 seconds) of interaction. During period 1, mother and infant engage in a spontaneous face-to-face interaction; in period 2 (the SF), the mother assumes a neutral facial expression, is nonresponsive and does not interact with the infant; in period 3 a typical interaction is resumed. During the SF, which represents a period of maternal unavailability, infants typically withdraw their gaze from the mother and stop smiling at her and increase their negative affect (Adamson & Frick, 2003).

Use of the SF procedure revealed the communicative and the regulatory functions of touch. In one study infants were found to manifest the SF negative effects only when touch was part of period 1 (Gusella et al., 1988), pointing to a communicative function of touch. Another study found that touch was used more often in the period 3 than in period 1 pointing to its regulatory function (Koester et al., 2000). In a modified form of the SF, if the mother is allowed to touch her infant during the SF period, the negative effects are drastically reduced. Indeed, infants are not distressed, they smile and make eye contact with the mother (Stack and Muir., 1990, 1992; Pelaez-Nogueras et al., 1996; Stack & LePage, 1996). These findings suggest that during negative emotional states touch helps the infant to regulate and is effective in conveying affective communication when other communicative channels are blocked. When these effects have been investigated further it was shown that the positive effects are afforded by the tactile and not the visual component of the stimulation: touch reduced the SF effects even when the infant could not see the mothers' hands and only seeing the moving hands had no effects (Stack and Muir, 1992).

Measuring touch during mother-infant interactions, during the SF paradigm and modifications thereof, revealed *1)* a pervasive presence of touch (across studies its presence was observed between 55% and 99% of total

interaction time; e.g. Jean et al., 2004; Stack & Muir, 1990; Stack & Muir, 1992) and *2)* that episodes of maternal touch have different functions (based on the measured infants' behaviour). However, while measuring the presence/absence and the duration of such episodes is important, studying the quality of the touch has an obvious value towards the understanding of its functions. To fill this need, a series of scales have been devised to best capture the specific forms of maternal touch and their roles (e.g. The Caregiver-Infant Touch Scale, Stack et al., 1996); The Touch Scoring Instrument, Polan & Ward, 1994); The tactile Interaction Index, Weiss et al., 2001; Functions of Touch Scale, Jean and Stack, 2009). These scales differentiate in the number of subcategories of touch they measure, with some being more fine-grained than others, but in general they all converge towards three main subcategories of interest: affectionate (e.g. kissing, caressing), stimulating (e.g. tickling) and instrumental touch (e.g. wiping the infant's mouth). While stimulating touch has usually been associated with social communication and regulation of affect, affectionate touch was usually employed by mothers to regulate the infants in case of distress. This work suggests that different forms of maternal touch have different functions. However, these functional classes can only be verified by experimental manipulations. In this direction, Stack and colleagues showed that not all touches are the same in reducing the SF effects and that gentle stroking (affectionate touch) as compared to static touch is more effective (Jean and Stack, 2009; Stack and Muir, 1990, 1992).

The idea that different touches subserve different functions has also been directly tested by asking mothers to elicit a number of behaviours in their infants. It was shown that depending on the behaviour they are prompted to elicit, mothers employ different types of touch. For example, high levels of stimulating touch (and low levels of affectionate touch) were used to maximize infants' smiling (Stack et al., 1996). This shows that with variations in instructions mothers changed their touch, and changes in touch were associated with measurable changes in infants' behaviour. These findings also suggest that during the first months of life infants may become sensitive to specific characteristics of their mother's touch, not only to its presence or absence (Stack and Muir 1992).

The aforementioned touch scales have been used to measure how patterns of maternal touch change across early development (e.g. (Ferber et al., 2008; Jean et al., 2009). Ferber et al. (2008) measured touch during both a caregiving and a play session (dyad on the floor with age appropriate toys) at different time points during the first year of life (cross-sectionally); they recorded a decrease in affectionate and stimulating (but not instrumental) touch between 6 and 9 months of age. A decline in use of affectionate touch between 4 and 12 months has also been previously reported (Crnic et al., 1983). Jean and collaborators followed a cohort of infants longitudinally across the first 6 months of life and manipulated the context of interaction (infant on the mum's lap without toys vs. dyad on the floor with age appropriate toys). Overall infants received more touch when on the lap vs. when on the floor, but this difference was due to the fact that in this context the mum had to provide the infant with support. Age related findings show a decrease of touch between 1 and 6 months and that affectionate touch is more abundant in the younger infants only in the lap condition (Jean et al., 2009). While these are interesting findings, the small sample size (n=12) invites caution. If the frequency of affective touch is normally distributed as the licking behaviour in rodents, larger samples are needed to reveal this frequency distribution.

Since the studies presented thus far always measured the effects of touch in the context of a dyadic interaction, it is difficult to disentangle the unique contributions that touch had on the observed effects from those associated with its agent (the mother). Being able to replicate similar effects outside of the interactive context would provide us with a better understanding of the functions of touch. A study that that represents a step in this direction is the recent work by Della Longa and colleagues where infants were sitting in a car seat in front of a screen and had no visual access to the agent of the tactile stimulation. In their work the authors investigated the idea that touch facilitates tuning to social signals using an habituation paradigm and showed that 4-month-old infants who were stroked on the forehead during the habituation phase, where the stimulus consisted of a face with averted gaze, learnt the identity of this face while infants who received either no touch or non-affective touch failed to discriminate it from a novel one (Della Longa et al., 2017). Thus, this work suggests that maternal touch similarly to other social signals, such as

direct gaze or infant directed speech (e.g. Senju & Csibra, 2008), can promote early learning. This study elegantly shows that one function of affective touch is to help infants orient to social signals and more studies of this kind, where the touch is stripped from its contextual cues, are needed in order to confirm those functions initially inferred via observation of naturalistic interactions.

Notwithstanding the magnitude of the research presented in this section, the mechanisms that underpin each of the proposed functions of social touch remain unexplained. Measuring the impact touch has on different systems offers a window into the understanding of such mechanisms. Eventually measuring how social touch impacts physiology (at different levels) will help us better define the border that separates social touch from other forms of stimulation. Notably, those touches that despite different physical properties have a similar impact on physiology will be grouped together under 'social touch'.

1.3.2 Neuroimaging studies of social touch

Neuroimaging studies of touch have also lagged behind those investigating early visual or auditory processing. There has been some sustained interest in the processing of touch in the primary (SI) and secondary (SII) somatosensory cortices. SI is located on the postcentral gyrus while SII is located on the parietal operculum. In adults, response in these cortices is typically contralateral to the side of stimulation (Fritsch & Hitzig 1870; Penfield & Boldrey, 1937), with ipsilateral activation sometimes reported as well (e.g. Hari et al., 1983; Nihashi et al., 2005). An important feature of SI is its somatotopic organization, which means that the body surface is mapped in a topographic fashion on the postcentral gyrus. This mapping is also referred to as Penfield's homunculus after the neurosurgeon who first described this organization (W. Penfield & Boldrey, 1937; W. Penfield & Rasmussen, 1950). While there is also somatotopic organization in SII this is less well defined and seems to be less fine-grained than in SI (Ruben et al., 2001).

Most of our current knowledge on the neural processing of tactile stimuli in infancy derives from studies run to answer mainly two questions: *1)* How early are adult like responses observed in the somatosensory cortex? And *2)*

How is pain processed in the infant's brain? Studies aimed at addressing the latter question often also contributed to answering the first one. Work on pain perception in infancy was initially motivated by an urge to understand how neonates in NICUs process the noxious procedures they are frequently exposed to. Indeed, it was suggested that if a cortical response to noxious stimuli can be measured in these infants it implies that they have a conscious sensory perception of pain (this remains to date a contentious issue), and that particular attention has to be dedicated to devising pain relief methods appropriate for this age (for an overview see Slater et al., 2007). In these experiments the response to a noxious stimulus (usually a clinically required heel lance or venipuncture) was often compared to the response to a (preceding) innocuous stimulus. Measuring neural responses to innocuous tactile stimuli in infants born preterm offered the possibility of knowing how touch is processed already during prenatal development. Using functional Near Infrared Spectroscopy (fNIRS), Bartocci and colleagues showed that non-noxious tactile stimulation (skin disinfection with a cotton pad applied to the hand) elicited bilateral activation over the primary somatosensory cortex from 28 weeks' gestational age (Bartocci et al., 2006). However, no response was detected with fNIRS following innocuous stimulation using von Frey hairs at intensities sufficient to elicit visible foot withdrawal (Slater et al., 2006). Tapping on the foot (non-noxious stimulation) instead generates evoked potential responses over central electrodes (Cz-CPz) (measured with the electroencephalogram- EEG) in infants born preterm (born 24-37 weeks) and these responses closely resemble those observed in infants born at term (Slater, Worley, et al., 2010)[12]. Functional magnetic resonance imaging (fMRI) has also been used to chart the development of somatosensory responses. In two studies conducted with preterm infants, one reported bilateral activation in SI following passive movement of the forearm (Heep et al., 2009) and the other showed contralateral SI responses to passive extension of the hand (Arichi et al., 2010). Thus, general tactile stimulation activates somatosensory cortex already prenatally.

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[12] In a recent paper, it was suggested that the reason why a haemodynamic response was not measured in Slater et al. (2006) is probably due to the stringent criteria that was set for detection of a response given the likely low signal-to-noise ratio (Verriotis et al., 2016).

As far as pain processing is concerned, contralateral haemodynamic responses to noxious stimuli (Bartocci et al., 2006; Slater et al, 2006) and nociceptive event related potentials (Fabrizi et al., 2011; Slater et al., 2010)have been reported in preterm born infants from the 24th gestational week. These works also showed that responses to noxious stimuli are larger than those to innocuous tactile stimuli. Furthermore, the younger the infants the more pronounced the response to pain which could explain the sensory impairments associated with preterm birth (e.g. Anand, 2000). Since pain is outside the scope of this thesis, these results will not be discussed further.

Measuring neural responses to tactile stimulation in full term newborns showed bilateral activation over the somatosensory cortex measured with fNIRS (Shibata et al., 2012). In their study, Shibata and colleagues measured activation to visual, auditory and tactile stimuli and found that the latter elicited the strongest and most widespread response. This difference in magnitude of elicited responses across sensory modalities could be explained by the fact the somatosensory system is the earliest to develop and that at birth it is more mature than other systems (Montagu, 1978; Atkinson and Braddick, 1982). Bilateral responses to tactile stimuli in full-term newborns have also been reported using magnetoencephalography (MEG) (Nevalainen et al., 2008) and fMRI (Erberich et al., 2006; Williams et al., 2015). Nevalainen and collaborators elegantly showed that the contralateral activation temporally preceded ipsilateral one in both SI and SII (Nevalainen et al., 2008).

Recent work showed that both lateralization and somatotopic organization of the responses emerge shortly after birth. Evidence of adult-like contralateral somatosensory responses to touch was observed using EEG and MEG already in 2-month-old infants (Meltzoff et al.,2018a) and in older infants aged 4 to 10 months (Meltzoff et al., 2018; Rigato et al., 2017, 2014; Saby et al., 2015). Given evidence for the lack of lateralization at birth (e.g. Shibata et al., 2012, Nevalainen et al., 2008; Erberich et al., 2006; Bartocci et al., 2006) these findings suggest that this response develops during the first two months of postnatal life. Alongside the early lateralization of somatosensory responses, evidence of somatotopic organization has also been reported using EEG in 2- and in 7-month-old infants (Meltzoff et al., 2018; Saby et al., 2015). Stimulation to the hand and to the foot elicit spatially distinct electrophysiological

activation that resembles the location described in the somatosensory homunculus. Furthermore, it has been shown that in infants the somatosensory cortex is involved not only in the personal experience of touch, but this sensory area is also vicariously recruited when seeing another person being touched. Vicarious responses to touch have been reported using EEG in 4-month-old infants (Rigato et al., 2017) and using MEG in 7-month-old infants (Meltzoff et al., 2018).

Taken together these findings show that cortical processing of tactile stimuli in sensory areas is already present during prenatal development and that the development of adult like features such as lateralization and somatotopic organization follows shortly after birth. Therefore, an infant is born with the ability to process touch, but whether she can tell apart the tactile episodes that positively impact development (e.g. massage or skin to-skin contact) from other touches (e.g. touch that mediates routine care) is a question that still remains unanswered.

The answer to this question must lie in activation outside of sensory SI/SII. Sparse efforts in this direction have been undertaken during the last 10 years. The two studies, presented below, investigated responses to touch in the orbitofrontal cortex (OFC) a region described as part of the "social brain", the network of brain regions involved in supporting social function and understanding others (Brothers, 1990). Following the well documented effects that skin-to-skin contact has had on developmental outcomes of preterm infants, Kida and Shinohara were one of the first to investigate the neural systems underlying the processing of gentle, pleasant touch (Kida & Shinohara, 2013). They used a two-channel NIRS system to record responses over the anterior prefrontal cortex (which includes the OFC), an area involved in processing rewarding stimuli and shown to be recruited for the processing of pleasant touch in adults (e.g., Francis et al., 1999; Gordon et al., 2013; Hua et al., 2008). Stroking of the hand with velvet (pleasant touch) was compared to stroking with wood (neutral touch) in 3, 6 and 10-month-old infants. Results showed that increased responses to pleasant vs. neutral touch were not evident until 10 months of age (Kida & Shinohara, 2013). A study previous to this, aimed at answering a similar question, measured prefrontal activation to touch with cotton, plastic and wood in newborns. An increased bilateral prefrontal

response was measured when strokes were applied with cotton to the forearm, and when plastic was applied to the cheek, relative to stimulation with wood (Saito, 2009). The authors conclude that cotton and plastic tactile sensations were more pleasant than wooden ones and suggest that the differences in activation based on region of application could be explained by early experience.

Despite the inconsistent findings, these studies represent the first attempts to reveal how infants discriminate touches with different 'rewarding values' beyond sensory areas. Conclusions are hard to draw (small sample sizes and lack of replications) but the data at hand suggest that the prefrontal cortex can discriminate pleasant from neutral materials at birth and near the end of the first year. Interestingly both studies highlight the importance of maternal gentle touch in infancy for development but none used human touch as their stimulus. If the pleasant touch employed in these studies is representative of 'social touch' then these findings suggest that for infants, social touch is rewarding. Evaluation of its rewarding value could be a way of discriminating social touch from more general stimulation. However, these studies employed non-human objects and we don't know whether human touch is always processed as rewarding or whether different reward values are conferred to different forms of interpersonal touch. Investigation of other brain areas and of different stimuli is necessary to gain an understanding of the neural processing specific to social touch and of mechanisms behind different touch functions.

Neuroimaging techniques have also been employed to understand whether maternal touch affects connectivity in the social brain. As behavioural research showed that maternal touch engages infants socially promoting responsiveness and engagement (Peláez-Nogueras et al. 1996; Jean et al., 2014) researchers asked whether it can also shape the development of those brain circuits that promote social functioning. Frequency of maternal touch measured during a play episode was found to positively predict both resting state activity in the posterior superior temporal sulcus (pSTS, key node of the social brain) and enhanced connectivity between this area and the medial prefrontal cortex (mPFC, another key node of the social brain) in 5-years-old children (Brauer et al., 2016). Although this study was not run with infants and it considered maternal touch in general without measuring differential effects of different

categories, it is important because 1) it reflects the increasing attention paid to the impact that maternal care through touch has on development and 2) it shows a new avenue for infancy research. From this work stems the question of whether the beneficial effects of touch measured in early social interactions (behavioural studies described above) could be supported, at the neural level, by recruitment of the social brain.

Returning to the first question that opened this thesis regarding mechanisms ('*how does social touch promote development?*'), we can ask whether the role that social touch has on social-emotional development is supported by activation of the social brain. To answer this question, activation in areas other than the OFC (e.g. STS) should be measured in response to social touch.

1.3.2 Impact of touch on the autonomic nervous system

Measuring the impact that touch has on the autonomic nervous system (ANS) can also help unveil mechanisms behind the different functions of touch. The ANS regulates bodily functions including heart rate, digestion, respiratory rate, pupillary response (etc.). It is composed of two branches, the sympathetic and the parasympathetic nervous system. These two branches have opposite actions, in general the sympathetic system activates a physiological response (e.g. increases heart rate) and the parasympathetic one inhibits it (e.g. decreases heart rate). Measures of ANS activity include skin conductance, heart rate and pupil dilation. Capturing changes in any of these measures associated with touch episodes would index that touch taps into the ANS to elicit its effects, and the direction of these changes would reflect what system is being recruited. As to the behavioural studies reviewed above, mechanisms can be questioned (also, but not exclusively) in terms of ANS activity. For example, is the regulatory effect associated with affectionate touch mediated by changes in ANS? In those studies that used the modified SF paradigm (Jean & Stack, 2008; Stack & Muir, 1992), does touch reduce the negative effects of the SF increasing parasympathetic activity and/or reducing the general sympathetic increase? Does touch regulate arousal (intended as the total level of activity within the ANS) to a level that is optimal for paying sustained attention to the caregiver

and thus ensure infant's engagement in the interaction. Assessing how different forms of touch differentially impact the ANS could certainly be a sensible way to understand its function in development, aided by the fact that these measures are non-invasive and can be measured also during a naturalistic interaction. Notwithstanding the advantages associated with this line of research, studies that in infants measured ANS activity in relation to touch are scarce, and the existing ones only measured heart rate (and not other indexes of ANS activity such as pupil dilation or skin conductance).

Originally, cardiac responses to sensory stimuli were measured as a component of the orienting reflex (OR). Sokolov suggested that any time that our organism detects a sensory stimulation it responds to it with changes at the autonomic, motoric and neurochemical level, depending on the nature of the stimulus. Novel, innocuous stimuli elicit an OR, while aversive and painful stimuli elicit a defensive reflex (DR) (Sokolov, 1963). The OR is viewed as the initial stage of information processing where the organism lowers its sensory thresholds to allow further processing of the stimulus. At the autonomic level, heart rate deceleration is a component of the OR and acceleration of the DR (Graham & Clifton, 1978).

Much of the early research that measured HR changes in infants was aimed at showing how the OR changes with development. This research used very simple stimuli (simple sounds, flash of light and brief touches). Compared to sounds and visual stimuli, however very few studies measured OR to tactile stimuli. These studies showed that foetuses respond to vibrotactile stimuli with cardiac accelerations (Kisilevsky et al., 1992), and this acceleratory response is present also at birth (Pomerlau, Gray and Crowell, 1968). The decelerative response typical of the OR was shown to develop during the first few weeks of postnatal life and to be in place by 11 weeks (Gray & Crowell, 1968). While these findings show that infants can detect touch at the autonomic level, these do not inform us on how touch is processed past the initial OR. For example with continuing stimulation, as would be the case in social interaction, is the deceleration sustained? Importantly these studies did not investigate whether different forms of innocuous touch elicit ORs of differing magnitudes, as it was suggested that factors such as novelty, the significance of the stimulus to the organism, and preference can predict the size and length of the cardiac

deceleration. For example infants respond with longer and larger HR decreases following visual stimuli they prefer (Lewis, Kagan, Kampbell, 1965).

Based on findings in adults and animals it was proposed that touch increases parasympathetic activity, as indexed by heart rate and blood pressure decrease. Specifically, such effects have been observed in response to static touch (touch on the wrist) in human adults (Drescher et al., 1985, 1980; Wilhelm et al., 2001) and to affiliative touch (hugging, stroking, massaging) in human adults (Ditzen et al., 2007; Grewen, Girdler, Amico, & Light, 2005; Light, Grewen, & Amico, 2005; Triscoli, Croy, Olausson, & Sailer, 2017a) and animals (petting in dogs: Lynch & McCarthy, 1969; allogrooming in monkeys: Aureli et al., 1999; Boccia, 1989; Grandi and Ishida, 2015; allogrooming and stroking in cows: Sato et al., 1993; Schmied et al., 2008; stroking in lambs: Coulon et al., 2015). The finding that across species affiliative or affectionate touch decreases heart rate is certainly interesting and offers a working hypothesis to be tested in human infants. Does social touch promote development via increasing parasympathetic activity? However, to date only one study measured heart rate changes in infants in response to different tactile stimulations. Nine-months-old infants were stroked on the arm with a soft brush at different speeds (slow, medium, fast) and it was hypothesized that if the medium velocity touch was perceived by the infants as the most pleasant in comparison to the other two stimulations, this would be reflected in larger heart rate decreases. The authors found that while slow and fast touch led to no changes in heart rate, the touch performed at a velocity that resembles a caress elicited heart rate decelerations (Fairhurst et al., 2014). In this study heart rate was averaged across the entire 10s of stimulation making it hard to draw conclusions about differences in OR (observed in the first 5 s following stimulation onset) and sustained responses across stimuli. While these findings are in line with works in animals and human adults and suggest that affectionate touch at the autonomic level decreases HR in human infants, it is to date an isolated study that needs replication.

There are two ways of assessing the impact of touch at the autonomic level; at baseline (as in Fairhurst et al., 2014), or by measuring how autonomic reactivity (in a stress inducing condition) is modulated by touch. The latter was tested in a study that used a modified SF paradigm with 6-months-old infants

(Feldman et al., 2010). The SF period has been shown to increase heart rate (Haley & Stansbury, 2003). Feldman and colleagues used the modified SF paradigm, where the mum is allowed to use touch during the SF and showed that touching the infant during the period of maternal unavailability increased heart rate variability (a measure of parasympathetic activity) as compared to the no-touch condition. Furthermore, this study showed that higher heart rate variability was associated with episodes of touch synchrony during the free play period (the matching of maternal affectionate touch with mother and infant mutual gaze) versus mys-synchrony (instances when the mother uses stimulatory and proprioceptive touch while the infant averts gaze). In this work, they did not investigate whether the use of one type of touch was more effective than others (e.g. affectionate vs. stimulatory) in regulating autonomic activity, as the results emerged from comparing the touch to the no touch group.

Further evidence exists that maternal touch modulates stress reactivity. Sharp and colleagues showed that infants who receive higher levels of maternal stroking (measured with a self-report measure) show increased heart rate variability during the SF paradigm compared to infants exposed to low maternal stroking (Sharp et al., 2012). This study aimed at assessing whether maternal touch can moderate the negative effects that prenatal depression has on HPA reactivity and on negative emotionality. Maternal depression was only significantly associated with increased physiological and behavioural stress reactivity in the presence of low maternal stroking. Notably, breastfeeding had no effect on this relationship, showing in line with findings with rodent models, the specificity of the link between dynamic stroking touch and stress reactivity (Sharp et al., 2012).

The existing evidence that social touch promotes development modulating the ANS is encouraging, although still scant. Importantly the studies conducted with human infants show that caressing-like stimulation decreases heart rate and that touch in general during a stressful situation regulates heart rate variability. It remains to be ascertained whether other forms of affectionate touch have the same effect on baseline levels of HR and whether the effects on stress responsivity can be narrowed to a specific touch type.

1.3.3 Impact of touch on the endocrine system

Besides the neural and the autonomic level, the question of how touch impacts development can be answered via measuring the impact of touch on the endocrine system. However, as with research using neuroimaging tools and indexes of ANS activity to quantify the effects of touch in development, the number of studies that have measured hormone changes associated with touch are also rather limited.

Understanding how touch modulates the HPA activity is of great importance if we want to understand whether touch regulates the stress response in human infants via mechanisms similar to those identified in rodent models. In preterm infants, it was shown that touch interventions did not have a clear effect on baseline cortisol levels but did have some effects on cortisol reactivity.

A few studies have measured how massage therapy affects cortisol levels in full term infants[13] and showed, again, inconsistent findings. Two studies reported that the massaged group has lowered cortisol levels compared to the non-massaged group (Field et al., 1996; Schanberg et al., 1996) and the other one showed instead an increase in cortisol following massage (White-Traut, 2009). A Cochrane review on the effects of massage in typically developing infants concluded that massage has no effect on cortisol levels (Bennett et al., 2013). In a study published after this review, massage was shown to decrease cortisol in a group of newborns with gastroesophageal reflux disease. During the six-weeks' intervention (two massages a week) cortisol was measured before and after each massage session, and it was shown that pre-treatment cortisol levels decreased as well over time, suggesting that the impact on the HPA was not limited to the time of the intervention (Neu et al., 2014).

In typically developing infants no studies have been carried out to assess long term effects of maternal care through touch on stress reactivity. However, the immediate effect of touch on cortisol reactivity was assessed by one study (introduced above in section 1.3.3). Since this study showed that the SF period during a modified SF paradigm was shown to increase cortisol levels (Lewis &

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[13] In general, massage therapy has rarely been studied with full-term infants. This might relate to weight gain not being a concern for full-term infants.

Ramsay, 2005), it offered a good paradigm to measure if touch can modulate this response. Feldman showed that 6-months-old infants that received maternal touch during the still face differed from the control group in their cortisol levels during both the SF period and the reunion period. While the control group showed a cortisol increase during the SF period which did not return to baseline upon reunion, in the touch group, maternal touch decreased the magnitude of the initial increase and it facilitated a quicker recovery to baseline levels (Feldman et al., 2010).

1.3.4 From social to caregiving-affective touch

This section has clearly shown that the endeavours in devising behavioural measures to understand the functions of touch in early development have not been paralleled by equal efforts in applying physiological measures to understand the mechanisms behind these effects. Findings from experiments that explored how touch is processed at the neural and autonomic level have shown us that as early as in the womb infants can process tactile stimuli (showing activation in the somatosensory cortex and an orienting response when they are exposed to touch). This means that at birth infants are capable of processing the wide range of somatosensory episodes that comprise a newborn's experience. However, these results pertain to somatosensory processing in general and do not help us answer the questions of whether and how social touch impacts development. There are only a handful of studies that have tried to pursue this line of research and showed that infants can discriminate pleasant from neutral touch at the neural and at the autonomic level, suggesting that pleasant touch is processed as rewarding (activity over the orbitofrontal cortex) and that it elicits parasympathetic activity (decrease in heart rate). Furthermore, it was shown that maternal touch can modulate stress responsivity both at the autonomic (increasing heart rate variability) and at the endocrine level (dampening cortisol reactivity). Thus, infants find touch with a soft material rewarding and possibly relaxing (but does this hold true if stroking with the human hand is used instead?) and mum's contact can help to manage a stressful situation (in line with animal models, thus suggesting conserved mechanisms). While encouraging, these findings come from only four

experiments, and certainly are not robust enough to support statements on the importance of touch in early development and to draw firm parallels with animal work.

Why have so few studies attempted to quantify effects of touch during early development via different physiological measures despite the extensive evidence from animal models, interventions in preterm human infants and behavioural studies in typically developing infants? Arguably, the *stimulus* is the problem that hindered research in this field. In order to answer the questions of whether and how social touch impacts early development we first have to agree on *what social touch is* in order to manipulate it. Research has not converged toward one specific form of interpersonal tactile contact as critical for development: while individual works from animal models and preterm human infants concluded that 'social touch' promotes development, each of these lines of research found effects using different stimulations. Thus, the term social touch has been used to indicate different forms of contact. Young mammals across species seem to benefit from contact comfort, licking and grooming, massage and skin-to skin contact. One could argue that the minimum common denominator that is shared across these different forms of social touch is that these signal the availability of a caring mother. Thus, social touch could be narrowed down to caregiving touch. However, we are still faced with the issue of defining the physical properties of caregiving touch in order to manipulate it as an independent variable. In addition, how does the human infant know how to discriminate caregiving touch from more general stimulation? Research I will review next suggested that there are low level mechanisms that allow the identification of affective touch and these are based on the functioning of a particular type of non-myelinated fibers in the skin. If we assume that caregiving touch includes affective touch, we can start by using an affective touch stimulus with clearly defined physical properties (see next section) to measure the effects of social touch on development. Thus, in this thesis I am measuring responses to *affective touch* which I consider part of *caregiving touch* within the broader category of *social touch* (Figure 1.3). Choosing affective touch does not rule out the possibility that other categories of somatosensory stimulation are of importance, it just provides a starting point to begin a systematic exploration of this seemingly forgotten sense.
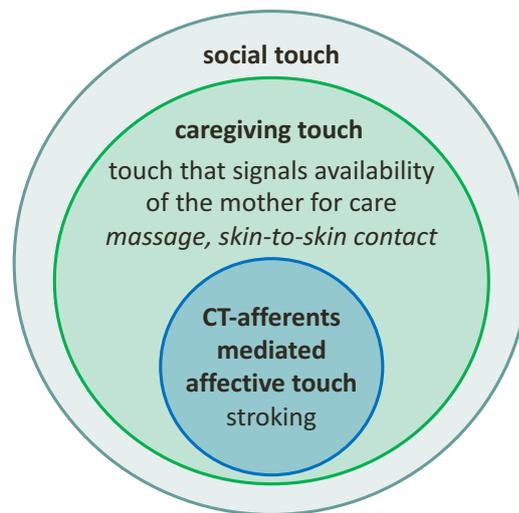
Figure 1.3 Diagram depicting subsets of social touch

1.4 Affective touch and CT afferents

1.4.1 C-Tactile afferents

For a long time the belief was held that humans have a quite uniform touch system. The sense of touch was thought to be mediated only by low-threshold mechanoreceptors (LTMs) found in the skin innervated by fast conducting, myelinated Aβ afferents (Mountcastle, 2005). When we haptically explore an object LTMs encode information on pressure, texture, vibration and slip and transduce it into nerve impulses in Aβ afferents. If mediated solely by this system, the function of our sense of touch is purely discriminative. However, using the microneurography technique[14] another class of afferents was discovered. These slowly conducting unmyelinated afferents which belong to the group of C afferents - classically described to mediate sensations of pain, itch and temperature, are known as C-Tactile (CT) afferents. CT afferents are around 50 times slower than Aβ ones. It was thus proposed that we have two touch systems, the fast (myelinated) and the slow (unmyelinated) one.

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[14] Microneurography is a technique that allows recording of the activity of single peripheral afferents on the skin. A recording device is connected to a tungsten needle electrode inserted in the skin. Position of the needle is manually adjusted until the electrode discriminates impulses from the nerve fiber of interest.

First evidence for the slow tactile system was presented by the Swedish physiologist Zotterman in 1939, who showed that light touch on the cat's leg produces impulses of different sizes, larger ones described as A and smaller ones, designated as C spikes. Zotterman hypothesised that the latter represent responses in unmyelinated afferents (Zotterman, 1939). This hypothesis was confirmed 20 years later using a saphenous cat nerve preparation with intact connection to the skin (Douglas & Ritchie, 1957, 1962). In their experiments, Douglas and Ritchie show a longer latency of the C compared to the A impulses and report that C impulses are conducted at 1 m/s, thus indicating that these are generated by unmyelinated axons. They also report that these afferents are abundant in nerves innervating the hairy skin of the cat. A rather interesting finding is that A and C afferents respond differently to velocity changes of the tactile stimulation (Bessou et al., 1971). While the impulse rate of tactile myelinated afferents increases monotonously with velocity of the stimulation (linear relationship), the impulse rate of tactile unmyelinated afferents first increases and then declines (inverted-U relationship).

After their discovery in cats, CT afferents have been identified in the hairy skin of other mammals (Kumazawa & Perl, 1977; Leem et al., 1993)but for decades researchers failed to identify C tactile responses in the human skin; it was believed that the C tactile system had vanished throughout evolution, an idea supported by the fact that C afferents were less numerous in rhesus macaques than in cats (Kumazawa and Pearl, 1977). Careful microneurography studies eventually revealed that the hairy skin of humans is also innervated by C afferents that optimally respond to innocuous touch stimuli. Evidence for CTs was first reported on the face (Johansson et al., 1988; Nordin, 1990) and later also on the upper and lower extremities (Vallbo et al., 1999, 1993). Despite extensive research CT afferents have never been identified in the glabrous skin (Johansson & Vallbo, 1979; Johnson et al., 2000, Vallbo et al., 1999; Wessberg et al., 2003; Löken et al., 2009).

Extensive study of the electrophysiological properties of CTs in humans led to the finding that these afferents show an optimal discharge rate to tactile stimuli that move on the skin at a medium velocity (between 3 and 10 cm/s) (Nordin, 1990; Vallbo., 1999) and at the human body temperature (Ackerley et al., 2014). Thus, the CT system seems to be velocity and temperature tuned to a

tactile stimulus that resembles a human caress. Psychophysics experiments revealed that participants rate as most pleasant those stimulations that optimally activate CT afferents and there are significant correlations between CT activity and estimates of pleasantness when speed and temperature of the stimulus are varied (Loken et al., 2009; Ackerley et al., 2014). Taken together, results from electrophysiology and from psychophysics experiments suggested that, in humans, the CT system subserves the processing of affective touch. While the Aβ system conveys factual information about distinct feature of mechanical events on the skin, the CT system "picks out" those feature of the stimulation that have affective relevance in order to provide further emotional processing (Morrison et al., 2010). It is from the early identification of these two distinct touch systems (the myelinated and the unmyelinated one) in human participants that Vallbo suggested that the functional role of the slow system is that of mediating affective touch (Vallbo, 1993, 1999).

Besides showing that CT afferents are tuned to gentle stroking, it was shown that interpersonal stroking is tuned to optimally activate CT fibers. Notably, participants were asked to stroke their partner's arm, their child or an artificial arm, imagining it belonged to another person or to a beloved one. Results showed that slower stroking velocities (always within 3-10cm/s) were used with partners and babies while participants applied a much faster touch to the artificial arm, even when instructed to think that the arm belonged to a beloved person, often exceeding the optimal CT speed range (Croy et al., 2016).

The discovery of CTs and the suggestion that in humans, touch perception is mediated by a discriminative system and an affective one, has fuelled a whole new area of research into the specific properties of affective touch.

1.4.2 Brain processing of CT-afferent mediated affective touch

Neuroimaging studies using fMRI revealed that CT optimal (affective) touch elicits a pattern of responses different from discriminative touch. In these experiments touch is administered with a soft brush and either speed or location of application are manipulated. Notably, gentle stroking (3-10 cm/s)

delivered to the hairy skin (affective touch), is contrasted either to fast stroking (>10cm/s) or to the gentle stroking applied to the glabrous skin (palm of the hand, known to lack CTs) (control stimulus). The first difference in the brain networks recruited by discriminative and affective touch can be identified in distinct cortical targets. A cortical target is the earliest synapse from the thalamic nuclei. While the first cortical target of discriminative touch is SI/SII, ample evidence has pointed to the posterior insula as the primary cortical target for CT afferents (Morrison et al., 2011a; 2011b; Olausson et al., 2002, 2010; Perini et al., 2015); for a recent meta-analysis see Morrison, 2016a). Patients who suffered permanent loss of large diameters afferents (including Aβ), offered the unique opportunity to study the brain correlates of CT optimal touch, un-shadowed by Aβ concurrent activation. These studies were the first to reveal that CT optimal touch activates the posterior insula but not the somatosensory cortices (Olausson et al., 2002, 2008). In line with these findings, subjects with a severe reduction in C fibers show a lack of insular activation in response to slow brush stroking (Morrison et al., 2011).

It was proposed that the posterior insula is a target for all unmyelinated C afferents which convey information about the physiological condition of the body (Craig, 2002). This putative idea is supported by electrophysiological findings that showed that electrical stimulation of this area in awake patients elicits sensations of pain, temperature changes and "innocuous tactile sensations" on the contralateral side of the body relative to the stimulated hemisphere(Ostrowsky et al., 2002, 2000; Penfield & Faulk, 1955; Stephani et al., 2011)It is however unlikely that activation of the posterior insula can alone mediate the hedonic content of affective touch. Indeed, activation in this region was not found to correlate with subjective ratings of pleasantness (e.g. Morrison et al., 2011; Perini et al., 2015). Instead, the hedonic content of affective touch could be processed in cortical networks that follow the activation of the cortical target. After cortical targets that are domain specific for touch, the signal is processed in highly interconnected cortical networks throughout the brain.

Several fMRI studies identified a network of areas specifically recruited by CT optimal touch beyond the posterior insula. These include the posterior superior temporal sulcus (pSTS), medial prefrontal cortex, ventrolateral

prefrontal regions of the inferior frontal gyrus (IFG), dorsoanterior cingulate cortex (dACC), orbitofrontal cortex and amygdala (Gordon et al., 2013; Mcglone et al., 2012; Voos et al., 2013). The involvement of pSTS in the processing of CT optimal touch was replicated using fNIRS (Bennett et al., 2014). Thus, it was shown that beyond the insula, key nodes of the social brain are involved in the processing of affective touch. Showing that in adults affective touch processing is supported by the social brain represents a solid ground for asking whether the role touch plays in socio-emotional development is also mediated by recruitment of this network. Some work has addressed this question and it is now presented in the next section.

1.4.3 CT-afferent mediated affective touch in development

1.4.3.1 Neuroimaging studies

Shedding light on the neural processing of affective touch in adulthood led researchers to ask how early in development affective touch elicits a similar response. A study that used fMRI looked at the development of these responses from childhood to adulthood found that posterior insula and a region of the middle temporal gyrus (MTG) extending into the pSTS were activated by affective touch as early as 5 years of age, with frontal areas only consistently activated in adulthood (Bjornsdotter et al., 2014). This study suggests that the neural mechanisms for processing affective touch are already largely established in school-aged children. The development of these responses from birth have begun to be explored recently but results are thus far few and inconsistent. One study of newborns used fMRI and reported that newborns show activation in the posterior insula in response to affective touch (Tuulari et al., 2017), however no control stimulation was used in this study. Beyond the insula, the investigators reported activation in SI/SII regions and, even if at an uncorrected threshold, in the superior temporal cortex (Tuulari et al., 2017). The other two studies that measured processing of affective touch in infancy used fNIRS. Given that with this technique it is possible to measure hemodynamic changes happening on the surface of the cortex, researchers have focused on measuring activation from the cortical areas that are part of the

network of regions identified by the adult fMRI literature. Specific attention has been paid to the pSTS region, a key node of the social brain, as both studies chose to locate channels over the temporal cortex (for details on the NIRS technique please see Chapter 2).

Of these two studies, one measured responses to affective touch from the left temporal lobe in 2-month-old infants. Results showed increased temporal lobe responses to slow compared to fast stroking in the left middle temporal gyrus extending into STS and in the insula (Jönsson et al., 2018). The other study measured activation over the left somatosensory and right posterior temporal cortices in 7-month-old infants but found no discriminatory response between affective and non-affective touch stimuli (slow brush stroking vs. static touch applied with a block of wood) in these regions (Miguel et al., 2017). These three studies are of great importance because they represent this new direction that research is taking, trying to understand how early on infants can discriminate affective from non-affective touch. However much more evidence needs to be collected before we can draw firm conclusions. These studies used different stimuli contrasts and did not record responses from both hemispheres. It is possible that in Miguel's study there was actually a differential response but in the unstudied region of the left pSTS. Differences across these studies (brain regions/stimulation/agegroup) emerge from Table 1.2. In this table I have added also the two studies described in section 1.3.2 given that they used a stimulus that can be identified as CT-optimal.

study	Age	Technique used	Brain regions measured	Social touch	Non-social touch	Location stimulated on the body	Areas with social touch selectivity
Saito et al., 2009	2-11 days	fNIRS	anterior prefrontal cortex	3cm/s stroking with cotton	3cm/s stroking with soft plastic	left ventral forearm/ left mid- cheek (between participants)	aPFC: Cotton>wood on forearm; plastic>wood on cheek
Kida and Shinohara, 2013	3,6,10 months	fNIRS	anterior prefrontal cortex	gentle stroking with velvet	gentle stroking with wood	left hand palm	aPFC only at 10 months
Tuulari et al., 2017	11-36 days	fMRI	ROIs selected: postcentral gyrus/ insular cortex	3cm/s brush stroking	/	right anterior shin	postcentral gyrus/ insular cortex
Jönsson et al., 2017	2 months	DOT	left temporal cortex	2cm/s brush stroking	20 cm/s brush stroking	right forearm	left middle temporal gyrus extending into STS / left insular cortex
Miguel et al., 2017	7 months	fNIRS	right temporal cortex/ left postcentral gyrus	8cm/s	tapping with a squared- shape piece of wood	right forearm	/

Table 1.2 Neuroimaging studies that investigated affective touch processing in infancy

These studies do not currently allow one to draw conclusions regarding whether in infancy social touch promotes social-emotional development via activation of the social brain. Experimental work in this thesis will try and further our understanding on the association between affective touch and social brain responses in infants.

1.4.3.2 Heart rate studies

To date only one study measured ANS activity to CT-optimal and suboptimal touches in infants. In their study (introduced above in section 1.3.3) Fairhurst and colleagues measured heart rate responses to brush stroking delivered on the forearm at three different speeds: 0.3, 3, or 30 cm/s. They measured heart rate decelerations only to the CT-optimal speed and thus concluded that 9-month-old infants are sensitive to CT-afferents mediated

affective touch (Fairhurst et al., 2014). Since they averaged responses across the entire length of stimulation (10s), their finding suggests that CT-optimal touch induces a sustained cardiac deceleration.

Work in adults suggests that CT-optimal and suboptimal touches elicit differential orienting responses (with CT optimal touch eliciting larger ORs than CT-suboptimal touch) (Pawling et al., 2017). Unfortunately, Fairhurst's study did not offer insight into differences at the OR level in infants. Therefore, future works need to i) replicate this finding, and ii) further understand whether differences in the early processing of touches exist in young infants measuring ORs.

1.5 The current thesis

In order to be able to answer the broader question of '*how does social touch promote development?*' we first have to ask, '*what defines social touch*?'. I advanced the idea that among the more broadly defined social touch, *caregiving touch* promotes development. However, the boundaries that separate caregiving touch from the remaining forms of social touch are not clearly defined. If we accept the assumption that affective touch belongs to the caregiving touch category, then we can employ CT-afferents mediated affective touch in our studies to start quantifying the effects of social touch in early development. Thus, identification of the CT system in humans offered to us a social touch stimulus with well-defined physical properties. In addition, CT-afferent mediated affective touch represents a good candidate for social touch since our species seems to have conserved throughout evolution a system specifically dedicated to its processing. Therefore, for the work in this thesis I defined social touch as CT-afferents mediated affective touch and I set to measure the impact of this stimulus on the organism, aiming to clarify through which mechanisms it promotes development.

In this thesis, I measured responses to affective touch (CT-optimal touch) and contrasted it to non-affective touch (CT-suboptimal). The choice of measuring responses to affective vs. non-affective touch does not exclude the possibility that development benefits from other forms of touch, but it serves as a valid starting point. While some research has been undertaken to understand

whether human infants are sensitive to affective touch, evidence is still scarce and inconsistent. Work from this PhD aims to clarify these findings and further our knowledge on how infants process affective touch. Specifically, I investigate the effects of affective touch at two levels: brain (chapters 3 and 4) and autonomic (chapters 5 and 6).

Chapter 3 presents Experiment 1, where fNIRS is employed to measure responses over the temporal cortex to different tactile stimuli in 5-month-old infants. A gentle stroke performed with the human hand (CT-optimal touch) is contrasted to stroking with an inanimate object that is colder than the human hand (CT-suboptimal touch). With this experiment, I aimed to investigate if affective (vs. non-affective) touch elicits activation in key nodes of the social brain network (pSTS and inferior frontal gyrus), as early as 5 months of age. If cortical specialization to affective touch has already developed, differential responses should be observed in these regions.

Chapter 4 presents Experiments 2, 3 and 4. In these experiments fNIRS is employed to measure responses to two different contrasting touch stimuli. In Experiment 2 a gentle stroke performed with the human hand (CT-optimal touch) is contrasted to stroking with an inanimate object that moves at a much faster speed (CT-suboptimal touch) in 5-month-old infants. Experiment 3 measures responses from a broader portion of cortex, extending into the parietal lobe, to slow (CT-optimal touch) vs. fast (CT-suboptimal touch) brush stroking in 7-month-old infants. Hypotheses for these two experiments are the same advanced for Experiment 1. Experiment 4 uses the same experimental design as Experiment 3 but measures responses in 10-month-old infants. The aim of this experiment is to measure whether the processing of affective touch undergoes developmental effects across the first year of life. If I fail to observe a differential response to affective touch in the hypothesized regions at 5 and 7 months, it might be that extensive experience of touch is crucial for this discrimination to take place. Should this be the case, I expect a differential response to emerge in the 10-month-old sample.

Chapter 5 presents Experiment 5, where the object of investigation shifts from the brain to the autonomic level. Electrocardiogram (ECG) is employed to measure infants' heart rate changes to slow (CT-optimal touch) and fast (CT-suboptimal touch) brush stroking in 6- and 9-month-old infants.

This experiment tests the hypothesis that affective touch increases parasympathetic activity, indexed by sustained heart rate decrease. Additionally, this experiment attempts to answer another question that links affective touch to attention: does affective touch promote focused attention? Specifically, since arousal has been linked to attention, I investigated whether affective touch can shift arousal (indexed by heart rate) to a level that is optimal for focused attention. To measure this, concurrently to receiving tactile stimulation, infants performed a visual attention task where the latency to disengage from a central stimulus and reorient to a peripheral one was measured. I hypothesized that if affective touch decreased arousal (heart rate) infants would show i) longer latencies to reorient to the peripheral stimulus and ii) longer latencies to disengage from the central stimulus.

Chapter 6 presents Experiment 6 where heart rate responses to slow and fast brush stroking are measured in 1 to 3-month-old infants. I explored responses in this very young sample to measure how early in life effects of touch on the ANS can be observed. If for human infants, as for animal models, touch is most important early in life, its effects should be evident in this age-group. In this experiment, I investigated differences in processing of affective and non-affective touch looking both at orienting and at sustained responses.

The approach common to all experiments presented in this thesis is that of isolating responses to affective touch in the absence of social cues in any other modality. The rationale behind this choice is that of revealing the specific contribution(s) of tactile stimulation on early development. Additionally, in these experiments the infants' initial state was not manipulated (e.g. via inducing stress) as I wanted to measure a baseline response to affective touch. Should this signature response be isolated it would then be of interest to investigate what external (social cues) and internal (infant's state) factors can modulate it.

The following theoretical questions (that stem from the question that opened this thesis 'How does social touch promote development?') are addressed in this work:

1. ***Do young infants exhibit cortical specialization to affective touch (human stroking) in the posterior superior temporal sulcus and inferior frontal gyrus? (Ch.3,4)***
    1.1. *Is a difference in temperature sufficient to elicit differential responses in these regions? (Ch.3, Experiment 1)*
    1.2. *Can differentiating affective and non-affective touch on more than one dimension facilitate discrimination? (Ch.4, Experiment 2)*
    1.3. *Do young infants display differential cortical responses to the stimulus contrast typically employed in work with adults: slow vs. fast brush stroking? (Ch.4, Experiment 3)*
        1.3.1 *Do infants process CT-optimal touch delivered through a brush in the same way as stroking performed with a human hand?*
    1.4. *How does the processing of affective touch develop across the first year of life? (Ch.4, Experiment 4)*

2. ***Do infants display differential cardiac responses to affective and non-affective touch? (Ch.5,6)***
    1.1. *Does affective touch promote focused attention, via a decrease in arousal (indexed by heart rate) in infants between 6 and 10 months? (Ch.5, Experiment 5)*
    1.2. *Are younger infants more likely to display differential cardiac responses to affective touch (Ch.6, Experiment 6)?*

Chapter 2

Methodological considerations

2.1 Electrocardiography

2.1.1 Introductory remarks

Psychophysiology is the study of the physiological activities that underlie psychological events. Heart rate is one of the earliest used measures in psychophysiological research. While Willem Einthoven invented the first electrocardiograph in 1901, physicians' interest for how 'heart sounds' changed in association with psychological states predates by many centuries the possibility to reliably measure such changes. Early psychophysiology studies conducted with adults using the newly introduced electrocardiogram (ECG) showed that stimuli eliciting differential emotional responses also elicited differential heart rate responses (e.g. Darrow, 1929; Lacey, 1959).

Heart rate has been widely used in developmental psychophysiology to study cognitive development. In a similar way to other techniques (e.g. looking times measures) heart rate measures have been employed to measure infants' discriminative responses. If different stimuli elicit heart rate changes that differ in magnitude and/or direction (a heart rate increase or decrease) then it is inferred that infants can discriminate between the stimuli. Differential responses can be measured very early in life (e.g. Bartoshuk, 1964) and even prenatally. Indeed, a study using this approach showed that already at the 37th gestational week fetuses can discriminate between the mother's and a stranger's voice (Kisilevsky et al., 2003).

A wave of early developmental psychophysiological studies that measured heart rate was interested in the ability of infants to show *an orienting reflex* (OR) (Sokolov, 1963). As soon as we attend to a sensory stimulus, depending on whether this is novel or aversive, our organism responds either with an orienting or with a defensive response. Sokolov suggested that anytime our organism detects a sensory stimulation it responds to it with a response (involving changes at the autonomic, motoric and neurochemical level) that depends on the nature of the stimulus. Novel, innocuous stimuli elicit an OR, while aversive and painful stimuli elicit a defensive reflex (DR) (Sokolov, 1963). The OR is viewed as the initial stage of information processing where the organism lowers its sensory thresholds to allow further processing of the stimulus. At the autonomic level, heart rate deceleration is a component of the

OR and acceleration of the DR (Graham & Clifton, 1966). Infants start showing the orienting reflex to a variety of sensory stimuli already at birth with heart rate decelerations being more consistently observed as age increases (see Figure 2.1) (Reynolds & Richards, 2008).
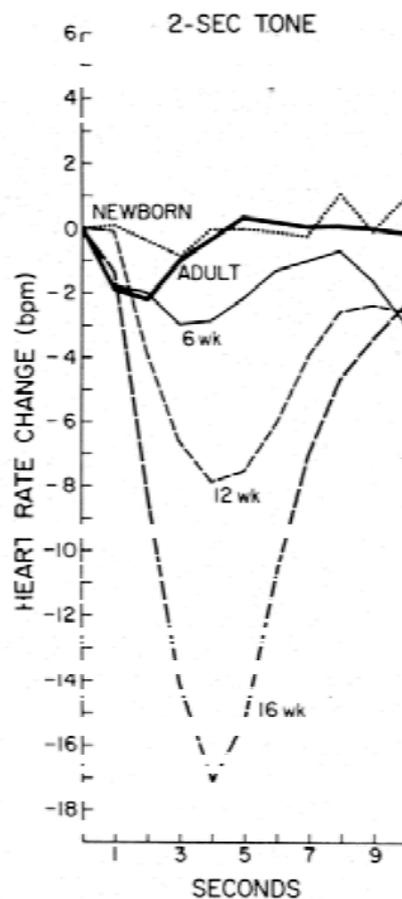
Figure 2.1 Example of the OR. Changes in HR response as a function of age to a 2s tone. From Graham et al., 1970.

Another line of work investigated *sustained heart rate decelerations* as a measure of attention. If a stimulus is presented for long enough and the infant voluntarily pays attention to it, the orienting reflex is followed by a sustained HR decrease that ends when the infant no longer attends to it. Heart rate has thus been used in infants to index the different attention phases (orienting, sustained attention and attention termination) (e.g. Richards & Casey, 1992).

In this thesis, I measured heart rate to investigate discriminative responses to a variety of tactile stimuli in young infants and I analysed both immediate and sustained components of these responses.

2.1.2 The heart

The heart is the strongest muscle in the human body. It is composed of three layers of tissue: the epicardium (outer layer), the myocardium (middle layer) and the endocardium (inner layer). The myocardium is where cardiac muscles are layered and these have properties that differ from those of the skeletal muscles found elsewhere in the body. The heart is divided into four chambers: the left and the right atria (which receive the blood from the lungs and the venous circulation) and the left and right ventricles (which receive the blood from the atria and pump it into the pulmonary circulation and the systemic circulation). The atrial and ventricular muscle fibers are mainly responsible for the pumping action of the heart. Cells of the cardiac muscle are connected to one another by intercalated discs, forming a functional syncytium. These intercalated discs contain two structures: *gap junctions*, that form ion channels between adjacent cells to allow for the depolarizing current to flow from one cell to the next, and *desmosomes,* that anchor tightly the cells to one another so they don't pull apart during contractions. The electrical coupling across the tissue, possible thanks to the gap junctions, allows a rapid spread of depolarization and the coordinated contraction of the entire heart that takes place in a rostral to caudal direction. The atria and the ventricles are connected by specialized cardiac muscle fibers that couple their pumping action, triggering ventricular contraction shortly after contraction of the atria.

Contractions of the heart muscle are what we refer to as heart beats. Each beat starts with depolarization of the sinoatrial (SA) and the atrioventricular (AV) nodes. The SA and the AV nodes are made up of specialized groups of cells that spontaneously generate an action potential. Once an action potential is generated at these initial sites it triggers the contraction of the heart spreading the electrical activity in a rapid and coordinated fashion to the four chambers. The SA node serves as the "pacemaker" of the heart since cells at this location have a resting membrane potential that is lower than those of the AV node, to allow the SA node to depolarize and repolarize at a much faster discharge rate, controlling the rate of the beat. The depolarization wave is spread from the AV node to the ventricles

through the bundle of His and the Purkinje fibers. Depolarization of the cardiac muscle happens in two phases, with a depolarization spike followed by a sustained depolarization (or plateau) that lasts for 200 to 300 ms before repolarization. This plateau phase results in a more sustained contraction and allows sufficient time for the ventricles to empty and refill prior to the next contraction.

2.1.3 The cardiac cycle and ECG

The term 'cardiac cycle' refers to the events that happen in the heart between each beat and the next. This is composed of two main phases, the diastole, during which the heart refills with blood, and the systole, during which the heart contracts and pumps blood. Electrocardiography (ECG) is the process of recording how the electrical activity of the heart changes over time as action potentials propagate throughout the heart during each cardiac cycle. Each event of the cardiac cycle is represented in the characteristic ECG tracing (see Figure 2.2) which comprises four waves.

Each cardiac cycle begins with the spontaneous firing of the cells in the SA node. In the ECG characteristic waveform, the P-wave represents atrial depolarization triggered by the activity in the SA node. Once depolarization reaches the AV node (Q-wave), this is spread to the left ventricle (R-wave) and to the right ventricle (S-wave). The completion of depolarization and the beginning of repolarization (marked by the onset of the T-wave) marks the end of the cardiac cycle. Thus, QRS complex reflects ventricular contraction and the onset of systole (Smith & Kampine, 1984). In this waveform two intervals are of importance. The first one, the P-R interval, represents the time required for the electrical impulse to leave the SA node and travel through the atria, AV node, bundle of HIs, and Purkinje fibers, while the second one, the Q-T interval, reflects the length of time it takes the ventricles to depolarize and repolarize.
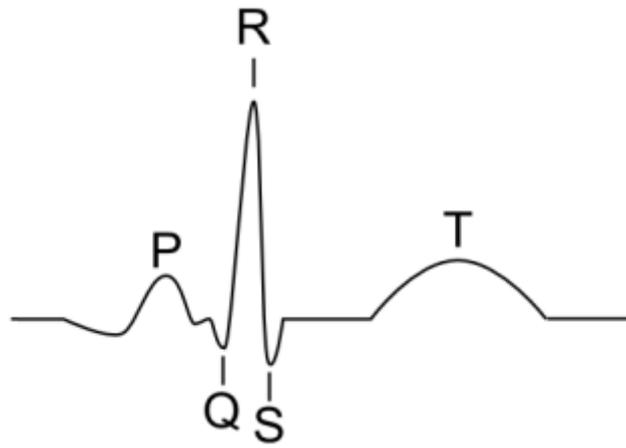
Figure 2.2 Components of the ECG tracing

2.1.3.1 The heart as a moving dipole

During waves of depolarization and repolarization ions flow across the cell membrane of cardiac muscle cells (through the *gap junctions*) generating voltage differences and currents on the surface of the muscle cell. When a cardiomyocyte is at rest (not electrically excited) the membrane potential is negative, with the voltage inside the cell being more negative than the outside. Inside the cell there are mainly negatively charged ions (Chloride, Ch-) and molecules (such as proteins), while outside the cell there is an excess of positively charged ions (such as sodium and calcium – Na+, Ca++).

An **electric dipole** is a pair of equal and opposite charges (*q+* and *Q-*). if these are separated by a distance *d* this becomes a dipole moment which is the measure of the separation of the positive and negative charges in a system of charges. This is expressed as *p=qd* where the moment (p) is given by the product of the charge (q) and the distance (d). In a resting muscle cell, the separation of charges across the cell membrane causes a dipole moment moving from the inside to the outside of the cell. The dipole moment is represented as a **vector** pointing toward the positive charges. (see Figure 2.3). However, since ECG measures voltage differences on the surface of the muscle cell, in a condition of rest the surface of a set of cells shows no electric dipole as it is evenly covered with an excess of positive charges. With depolarization, positively charged ions start flowing inside the cell and the voltage inside the cell is now positive compared to the voltage outside which is negative (the

direction of the vectors inverts). When the action potential is reached, depolarization propagates across adjacent cells since the potential difference at one side of the cell causes the adjacent cell to allow ions in. This depolarization wave creates an electrical wavefront with polarized cells at the front (positive surface charges), followed by depolarized cells behind (negative surface charges). This results in an electric dipole on the surface of the cardiac muscle pointing in the direction of the wave of depolarization (see Figure 2.3c). In reality, multiple waves of depolarization occur simultaneously, each with a slightly different orientation, and a correct representation would show multiple vectors. However, the ECG represents the heart as a single moving dipole depicted as a single vector, which is the mean of all the individual electrical vectors at any point in time. This moving dipole generates electric fields, which are detected by placing electrodes on the surface of the skin surrounding the heart. ECG electrodes are placed around this mean vector in pairs composed by a positive and a negative electrode and measure the voltage difference occurring between them. When the cardiac muscle is at rest no potential difference is recorded between the two electrodes, whilst during depolarization and repolarization potential differences are recorded. By convention, a wave of depolarization heading toward the positive electrode is recorded as a positive voltage (upward deflection in the ECG tracing) and a wave of depolarization travelling away from a positive electrode is recorded as negative voltage (negative deflection).

a)

+ + + +	+ + + +	+ + + +	+ + + +
- - - - -	- - - - -	- - - - -	- - - - -

b)

- - - - -	- - - - -	+ + + +	+ + + +
+ + + +	+ + + +	- - - - -	- - - - -

c)

Depolarized cells

SA node

Wavefront of electrical activity

Net dipole ·

Atria

Ventricles
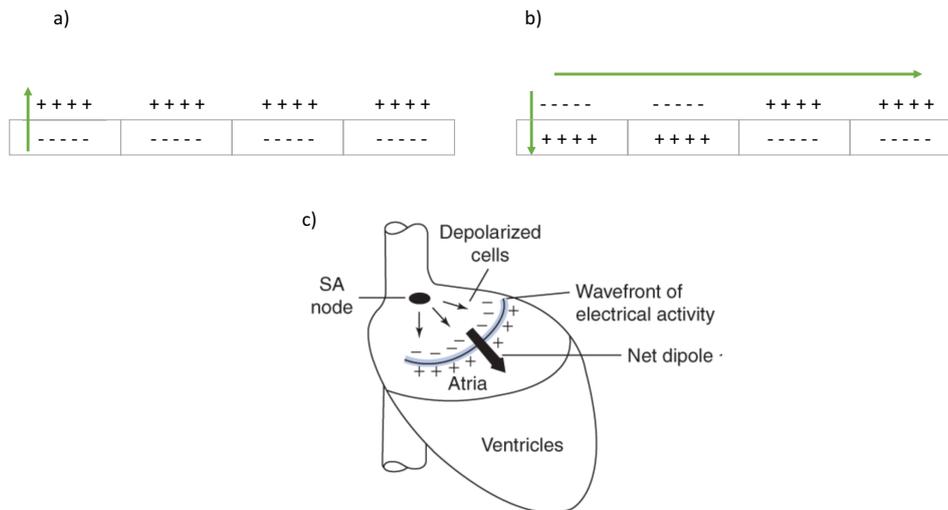
Figure 2.3 a) set of cells at rest b) set of cells where a depolarization wave propagates from the left to the right. Dipole moments are represented by green arrows. c) representation of net dipole moment of the heart during a depolarization wave

2.1.3.2 Recording ECG using a lead II configuration

The classic method of measuring ECG is to record from three electrodes (two measuring ones and a reference one) placed on three extremities of the body. The placement of the two measuring electrodes is normally based upon Einthoven's equilateral triangle (Einthoven et al., 1913), a configuration of leads that records the activity of the heart within a 2D geometric figure (Brownley et al., 2000). A common placement of the two electrodes is right arm/left leg. Based on Einthoven's terminology, this configuration is referred to as *lead II*. A lead is an abstract concept and refers to the source of measurement of the mean vector. Einthoven's leads are *bipolar* as they compare the electrical signal recorded from two electrodes. Lead II is the voltage between the (positive) left leg electrode and the (negative) right arm electrode. When using this configuration, the reference electrode is placed on the right leg; this electrode only grounds the signal reducing noise interferences, (it doesn't record anything on the trace).

For use with infants the location of the electrodes is normally shifted from the limbs to the chest/abdomen area (optimal recordings can be obtained also by placing electrodes on the back). This method is advisable as it is less

sensitive to movement artifacts produced by the movement of arms and legs. Figure 2.4 shows electrodes placed in a lead-II position with the negative electrode on the right chest, the positive electrode on the left abdomen and the reference on the right abdomen.
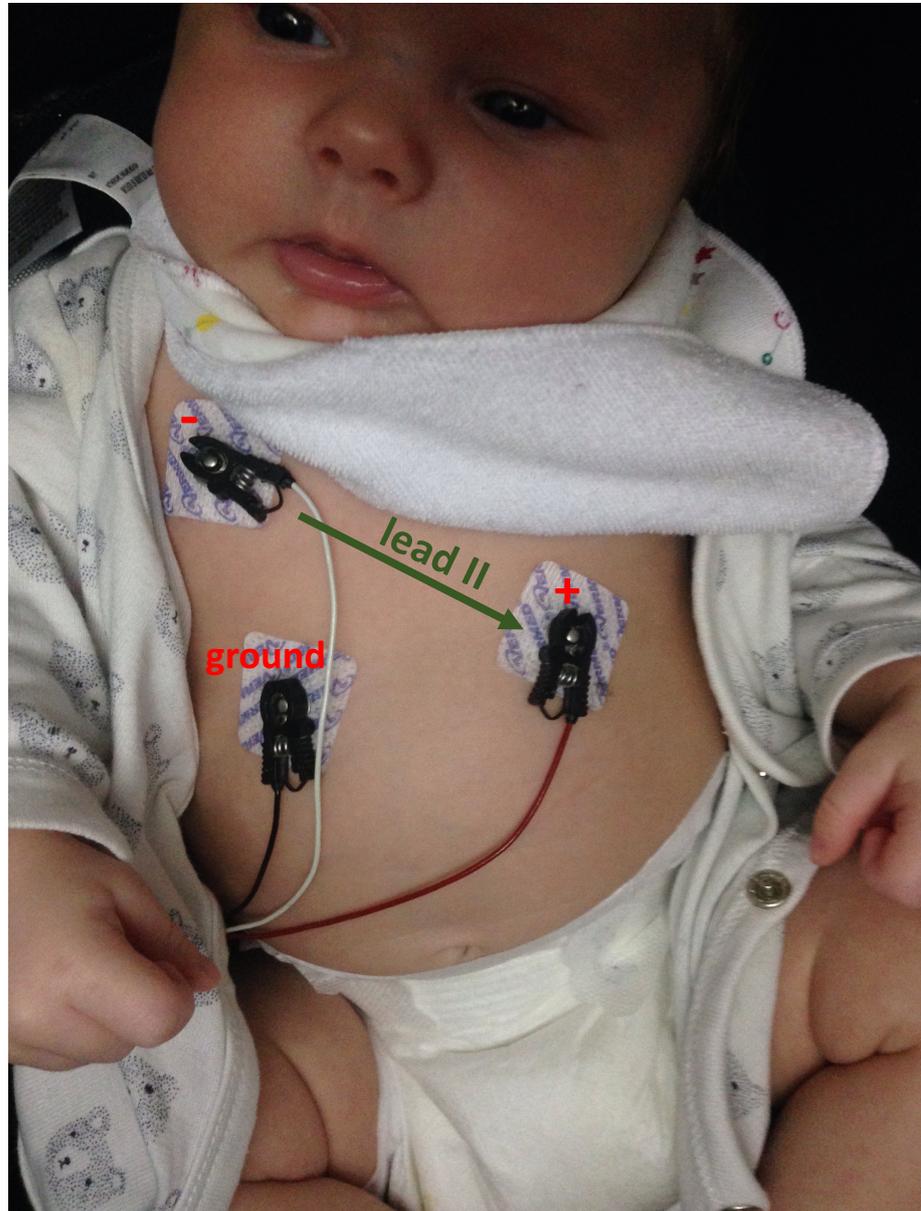
Figure 2.4 Picture of an infant that took part in our research to show electrodes placement used for experiments in the current thesis. Lead II is shown in green. The negative and the positive electrodes are indicated by red symbols.

2.1.4 Quantification of HR and approaches to analyses

Despite the fact that ECG provides us with information relative to each event that occurs during the cardiac cycle, what psychophysiological research mostly uses is a measure of the length of the cardiac cycle. This length is referred to as "inter-beat interval", IBI, and is expressed in milliseconds (ms). IBIs can be calculated by measuring the distance between one ECG waveform and the next by selecting one component as a target; each component of the wave (P, Q, R, S, T – see Figure 2.2) could in theory be used but it is common practice to use the peak of the R wave when measuring the length of each cardiac cycle. This is because the depolarization of the ventricles is recorded as the largest positive deflection on the ECG tracing, making the peak of the R-wave easy to detect amongst the other components. IBIs measured this way are also referred to as R-R intervals.

Heart rate (HR) is the number of beats over a certain amount of time and its units are beats-per-minute (BPM). HR can be calculated as the inverse of the IBI (6000/IBI in ms). For the current thesis data are expressed as IBIs and when using the term 'heart rate' I will be referring to IBIs (longer IBIs reflect an increase in heart rate, shorter IBIs reflect a decrease). IBIs can be averaged during certain periods of time to measure either baseline heart rate or changes (e.g. in response to a task). Alternatively, IBIs can be used to measure heart rate variability (HRV), where the variance of the IBIs is used for either time-domain or frequency-domain analyses. The sources of variability observed in HR are due to both central and autonomic nervous systems. A variability in HR typically measured in infants is respiratory sinus arrhythmia (RSA) which is the variation that naturally occurs during a breathing cycle and is under the influence of the vagus nerve. Since the vagus is a component of the parasympathetic system RSA is typically used to measure parasympathetic activity and can be referred to as vagal tone (Porges, 2001).

The choice of measuring HR or HRV depends on the research question and on the experimental design. Reliable measure of HRV requires minimum 1 minute of continuous recording (for a recent review on guidelines for research using HRV see Laborde et al., 2017). The typical structure for HRV experiments involves measurement of HRV at three time points: baseline (resting HRV),

event (reactivity HRV) and post-event (recovery HRV). An example of this experimental design is the still face paradigm described in the introduction[15]. Experiments where one or more conditions are presented in blocked trials, and trial length is typically shorter than a minute, do not allow the measurement of HRV. With such experimental designs, HR changes (relative to a baseline period) are instead calculated. For example, Fairhurst and colleagues used this approach to measure HR changes in response to three touch conditions (Fairhurst et al., 2014). It is possible to either average IBIs across the entire trial, or to average IBIs over shorter segments to look at orienting and sustained responses separately. Studies in this thesis (Chapters 5 and 6) have been designed to measured HR because I wanted to compare differential responses to stimuli within subject and to investigate differences in both orienting and sustained response to affective vs. non-affective touch.

2.1.5 Data Acquisition

The procedures of data collection, pre-processing, and analysis described in this section were common to all ECG research conducted as part of this PhD thesis. All ECG data were acquired at the Baby Lab of the Centre for Brain and Cognitive Development, Birkbeck, University of London. The studies in Chapter 5 and 6 were conducted using the same ECG acquisition system.

2.1.5.1 System used at CBCD

The ECG signal was measured using a Biopac wireless Nomadix system (BIOPAC Systems, Inc., Goleta, CA) with a sampling frequency of 1000 Hz. Both a low-pass (cutoff frequency: 35 Hz) and a high-pass (cutoff frequency: 1 Hz) filter were applied to the data online during the recording. A BioNomadix wireless transmitter was connected to the three lead wires. The lead wires clip onto disposable cloth base electrodes. We used pediatric silver-silver chloride (Ag/AgCl) electrodes with adhesive solid hydrogel. To ensure good contact of the electrodes parents were asked not to use any oil on the infant's chest prior

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[15] Feldman et al., 2010 measured HRV during the still face paradigm and had 3minutes baseline, 2 minutes SF, 2 min reunion for a total of 7 minutes.

to their visit. AcqKnowledge software package was used to record ECG signal and to analyze the data.

2.1.5.2 Overview of the testing procedure

All ECG sessions were conducted by the thesis author assisted by a student volunteer. Once the infant was ready, the author placed the three ECG stickers on the chest/abdomen using a lead-II configuration. Depending on the study, infants were placed either in an infant seat (Bumbo®)or in a car seat and the parent was always sitting behind them out of their sight. The ECG monitor was connected to the lead wires and secured either in the Bumbo or in the car seat out of infants' reach, then the AcqKnowledge software was started. If the author judged the quality of the signal being acquired good (evidence of clear QRS complexes) and if the infant was comfortable in the new setting the study would begin.

The experimental sessions lasted until the infant became fussy, otherwise if the infant was focused, the experimenter continued the stimuli presentation in order to increase the number of collected trials. The infant's behavior was recorded throughout the session using a remote-controlled video camera placed above the stimulus screen. Upon completion of the study the ECG stickers were removed using an oil spray to aid their removal and not hurt or irritate the skin.

2.1.6 Data analysis

Date analysis involved several data processing steps, which are described in detail in the following sections. The initial signal processing was carried out with the AcqKnowledge software package and with custom-built MATLAB scripts.

2.1.6.1 Video coding for data reduction

Infant's behavior was video recorded throughout the experimental session for offline coding. If an infant cried or yawned during a trial, that trial was marked and removed from further analysis since these behaviors modulate heart rate and thus mask effects due to the experimental manipulation. Movement was

also coded during these videos. A number from 1 to 5 was assigned to each subject with 0 reflecting no movement and 5 a great deal of movement throughout the experimental session. This variable was then used during further analyses to control for potential effects of movement on the response.

2.1.6.2 Preprocessing

The first step consisted of identifying and marking R-wave peaks on the ECG tracing. A function in AcqKnowledge was used to identify the peak of the QRS complex. This function requires one to input a threshold value in millivolts, so that only peaks that are above that value are marked as R-wave peaks. Following identification, the trace was visually inspected to check that only real R-wave peaks were marked. If markers were added to a noisy part of the signal rather than a peak, they were manually removed. On the other hand, if some peaks happened to be smaller than the threshold value (but the QRS complex was clearly visible) and would not be identified by the function, markers were added manually. In cases of missing peaks (segments where the ECG tracing is flat) or peaks not easily identifiable (usually in noisy segments), no peaks were added manually at this point. Once the inspection was completed, timestamps of the R-wave peaks were exported in a spreadsheet. Each of these stamps would reflect the occurrence of a heartbeat. Timestamps of the event markers that marked the beginning and end of each trial were also exported in the same spreadsheet.

2.1.6.3 Calculation of IBIs

At this point, timestamps (in ms) of the R peaks were exported from the AcqKnowledge software into Excel to compute IBIs and implement missing peaks. IBIs were obtained calculating the difference between consecutive R peaks. A missing peak would be indexed by a larger than usual IBI. If an IBI was more than 2 times bigger than the following one, this interval would be halved and a new timestamp added between the two existing ones. In case more than two consecutive peaks were missing no peaks were implemented manually. Alongside timestamps of the R peaks, timestamps that mark the beginning and end of each trial are exported from AcqKnowledge. Using custom built MATLAB

scripts, for each trial IBIs were averaged across segments of a predefined length (1 or 5 seconds, see individual chapters for specific details).

2.1.6.4 Data reduction

If a trial lacked more than 1/3 of beats due to recording or movement artifacts, it was discarded from further analysis. Trials were also removed if video coding indicated that the infant was crying or yawning during the trial. The minimum number of artifact-free trials was 2 per condition, across studies. Specific details regarding the numbers of trials included in each experiment are reported in the relevant experimental chapters.

2.2 Functional Near InfraRed Spectroscopy

2.2.1 Introductory remarks

Functional near-infrared spectroscopy (fNIRS) is a neuroimaging technique that in recent years has been increasingly used to study infant brain and cognitive development (for reviews see: Lloyd-Fox et al., 2010; Wilcox and Biondi, 2015; Aslin et al., 2015). fNIRS was used for the first time to measure functional activation of the infant's brain 20 years ago (Meek et al., 1998). Since this pioneering experiment, that used one channel and a simple visual stimulus, this technology has advanced to a level such that researchers can now turn to fNIRS to answer more complex questions on cognitive development recording responses from different brain areas simultaneously (Lloyd-Fox et al., 2010). One promise that fNIRS held is that it offered the possibility to measure the hemodynamic response which until then could only be measured with fMRI. While measuring a similar response, fNIRS overcomes the challenges that fMRI poses to infants' research (such as immobility of the subject, noise and length of the experimental session due to its low temporal resolution) and allows the testing of infants while they are awake, typically sitting on a parent's lap and relatively free to move.

Compared to the previous century, when neuroimaging studies with infants mainly relied upon the use of EEG, we are now able to further our understanding of cognitive development complementing the measure of the electrical activity of the brain with the measure of its functional activation.

When compared to two other techniques used with infants (EEG and fMRI), fNIRS has several advantages. Relative to EEG, the major advantage of fNIRS is that it offers a much higher spatial resolution allowing the localization of brain responses to specific cortical regions. In addition, as compared to fMRI, fNIRS has a better temporal resolution (the sampling rate of fMRI is around 0.5 hertz whereas that of fNIRS can be up to hundreds of hertz) and while fMRI only measures changes in deoxy-haemoglobin, fNIRS measures both oxy- and deoxy-haemoglobin. Thus, fNIRS provides a more complete measure of the haemodynamic response. Another advantage that fNIRS holds relative to both EEG and fMRI is that it is less sensitive to motion artifacts. Besides advantages, there are also limiting factors of fNIRS. Indeed, it has a much lower temporal

resolution than EEG (the sampling rate of which can reach up to a thousand hertz) and a lower spatial resolution compared with fMRI. Furthermore, fNIRS can only measure responses from the surface of the cortex, thus providing us with no information regarding activation in deeper structures.

2.2.2 General principles and methods of measurement

fNIRS takes advantage of the relative transparency of biological tissue to near infrared light (650-1000nm), so light in this part of the spectrum can travel through several centimeters of tissue without being absorbed. Since the spectral absorbance of oxy- and deoxy-haemoglobin is different in this 'optical window', near infrared absorption spectroscopy methods can be used to non-invasively measure tissue oxygenation.

In fNIRS, near-infrared light emitted from a source located on the head, travels through the scalp, skull and into the brain and is then detected by a detector placed in proximity of the source. At the detector level, changes over time in reflected near-infrared light are measured and used to quantify changes in blood oxy-hemoglobin (HbO_2) and deoxy-hemoglobin (HHb) in the underlying cortex. The concentration of HbO_2 and HHb depends upon the status of activity/inactivity of the brain area we are measuring from. Neural activation in response to a task is accompanied by higher metabolic demand which results in higher blood flow. During activation, local concentration of HbO_2 will increase (to supply oxygen to the active brain region) while that of HHb decreases (as it is displaced from the veins). Thus, changes in HbO_2 and HHb measured with fNIRS are used to infer localized brain activity.

In this thesis, I used fNIRS to measure differential responses to different tactile stimuli over the temporal cortex (Chapters 3 and 4).

2.2.3 Absorption of light in tissue and the Beer-Lambert law

When near-infrared light emitted by a source travels through tissue, only a fraction of its initial amount is picked up by the detector; the rest of it is either absorbed or scattered by the tissue. This loss is referred to as *attenuation*.

Human tissues contain a variety of substances that absorb light and whose absorption spectra at near-infrared wavelengths are well defined. These are present in sufficient quantities to effect measurements of transmitted light. The concentration of most compounds (e.g. water, melanin, bilirubin) remains stable over time, therefore when measuring light attenuation, these are treated as constants. Instead, the concentration in tissue of absorbers as HbO_2 and HHb varies with tissue oxygenation and metabolism.

Each compound has an absorption coefficient (μ_a) that describes how common an absorption event is (it represents the average distance light will travel through a material before it experiences an absorption interaction). This coefficient depends upon which wavelength is used (it is this variation in the absorption coefficient with wavelength that makes optical spectroscopy very useful to measure blood oxygenation). The *Beer-Lambert law* describes absorption of light intensity in a non-scattering medium (see Figure 2.5). The law states that, absorbance of light (I_{out}) in a material is proportional to the thickness (x) of the material and to the concentration (c) and the absorption coefficient (μ_a) of the absorbers present in the material.

The law is expressed with this equation:

$$I_{out} = I_{in}\ e^{-x\,c\,\mu a}$$
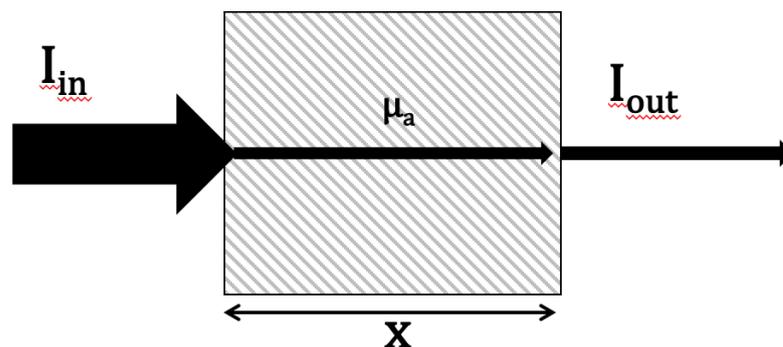
Figure 2.5 Absorption of light intensity in a non-scattering medium as described by the Beer-Lambert law

The absorption coefficient of a tissue is given by the sum of the absorption coefficients of the compounds present in the tissue (so $c*\mu_a = c_{HbO2}*\mu_{HbO2} + c_{HHb}*\mu_{HHb} + c_{water}*\mu_{water}$ ...)

A compound which absorbs light in the spectral region of interest is known as a *chromophore*. Each chromophore has its own particular absorption spectrum which describes the level of absorption at each wavelength. The absorption spectra for haemoglobin at the near infrared wavelengths can be seen in Figure 2.6.
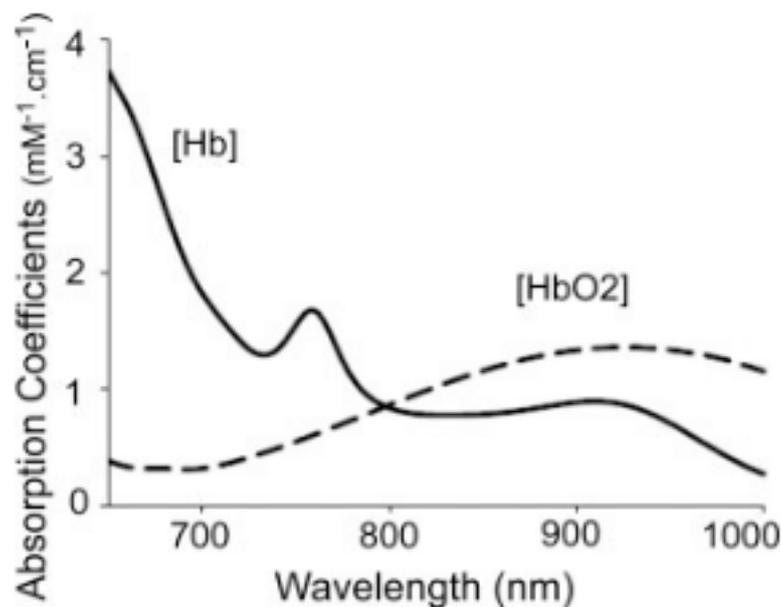
Figure 2.6 Absorption coefficient spectra for for HHb and HbO_2 in the near infrared region.

The absorption spectra of HHb and HbO2 remain significantly different in this near infrared region, allowing spectroscopic separation of the compounds to be possible using only two wavelengths. The wavelengths at which HbO_2 and HHb have the same absorption coefficients are known as isobestic points. The isobestic point at 800 nm (which can be seen in Figure 2.6) is especially important as two-wavelength NIRS system use wavelengths at either side of this point to estimate blood oxygenation (Everdell et al., 2005).

Therefore, one wavelength has a higher absorption coefficient value for HHb than HbO_2 and the other wavelength has a higher absorption coefficient for HbO_2. The wavelengths of the NIRS systems used in this thesis are described below.

2.2.4 Scattering of light in tissue and the modified Beer-Lambert law

Absorption only explains about 20% of total light attenuation: the remaining 80% of light is lost due to scattering. During scattering the direction of near-infrared light changes, while its energy remains unaltered. Thus, the main effect of scattering is the increase of the distance travelled by photons before being absorbed. These direction changes occur every time that the light moves through regions with different refractive indices (Elwell, 1995). Such variations occur, for example, at boundaries such as cell membranes or between bone and soft tissue. Each compound has a scattering coefficient (μ_s') that describes how common a scattering event is. Scattering coefficients in human tissue are roughly one hundred times greater than those for absorption (Cheong, Prahl, & Welch, 1990), which explains the aforementioned predominance of scattering interactions.

Given that the original Beer-Lambert law described absorption of light intensity in a *non-scattering medium* it must be modified for use with biological tissue, where scattering is prevalent (Cope & Delpy, 1988). The modification includes both an additive term, G, to account for scatter losses and a multiplier, to account for the increased optical pathlength due to scattering. Indeed, as shown in Figure 2.7, the true optical distance doesn't coincide with the geometrical distance of the medium (x). The actual distance travelled by the light is known as the differential pathlength and the scaling factor as the differential pathlength factor (DPF).
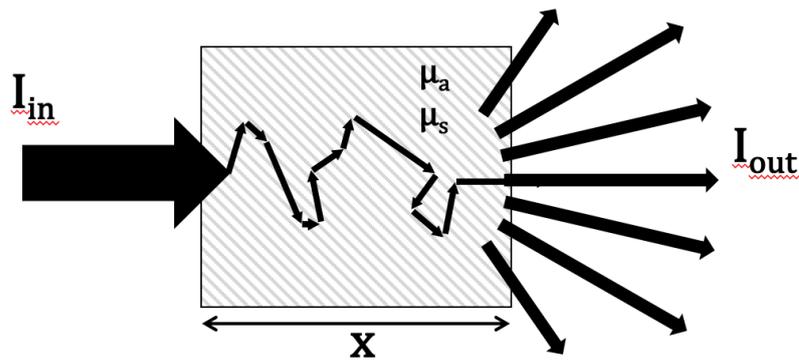
Figure 2.7 Absorption of light intensity in a scattering medium.

The modified Beer-Lambert law is expressed as:

$$A = \log_{10} [I_{in}/I_{out}] = (\mu_a . c . x) . DPF + G$$

$$(A = attenuation)$$

Losses due to scattering (G) are unknown since they cannot be accurately measured. Because of this we cannot calculate the *absolute* concentration of a chromophore. Instead we can assume that G remains constant during the fNIRS recording and, taking advantage of the fact that μ_a changes with time, we can calculate the *change* in concentration of the chromophore over time. Instead of an absolute attenuation we will obtain a *differential attenuation* expressed as:

$$A_{t1-t2} = \log_{10} [I_{out\,t1} /I_{out\,t2}] = (\mu_a\,c_{t2} - \mu_a\,c_{t1}) . x . DPF$$

Differential pathlength factor is complex to calculate since it depends on several factors including tissue type, wavelength used, absorption coefficient and the distance between source and detector. Amongst the methods available for measuring the optical pathleght, a common technique involves modulating the intensity of the light and measuring the phase delay that occurs when the light travels through the tissue (Lackowicz and Berndt, 1990). This phase shift can be converted into the distance that the light has travelled to obtain the optical pathlength. DPF measurement has been extensively investigated in

adults and infants (Duncan et al. 1995, 1996; Benaron et al. 2000). The DPF value is higher in adults (in the head is approximately 6) compared to newborn infants (app. 5) as the scattering coefficient of a neonate is lower (Duncan et al., 1995). In newborns Duncan et al. (1995) measured the DPF using 4 different wavelengths (690, 744, 807 and 832nm) and found that it ranged from 4.67 – 5.38 as the wavelength of light became more visible.

The choice of the correct DPF value is critical since using the wrong value will lead to inaccurate estimates of oxy- and deoxy-haemoglobin concentration changes. In order to choose the correct DPF for the work presented in this thesis we were guided by a study that developed an age-dependent formula to calculate DPF (Duncan et al., 1996).

2.2.5 Instrumentation- Continuous wave (CW) systems

In this thesis data in Chapter 3 and 4 have been collected using continuous wave (CW) systems. This is the most commonly used and simplest method used for functional infant activation studies.

CW are dual-wavelength systems that use two wavelengths (emitted from continuously emitting sources) in the near infrared region to measure changes in HbO_2 and HHb concentrations (Hebden, 2003). The choice of the two wavelengths is of great importance, as on it depends the accuracy of HbO_2 and HHb measurements (Lloyd-Fox et al., 2010). Ideally a pair of wavelengths should take into account cross-talk (contamination of oxyHb and deoxyHb signals by one another) and separability (differential noise effects on the signal at different wavelengths). While in in most systems the highest of the pairs is between 830 and 850 nm, the lower wavelength has been subject to a considerable amount of debate (Lloyd-Fox et al., 2010). Studies with adults suggested that 690-850nm (and wavelengths that approximate these values) is an optimal pair (Boas et al., 2004; Sato et al., 2004; Strangman et al., 2003; Yamashita et al., 2001). In a theoretical study, Uludag et al. (2004) used model-based estimates of cross-talk and separability to asses all combinations of two wavelengths between 610 and 920 nm. They conclude that cross talk is low and separability high if one wavelength is below 720 (or between 750 and 770) nm and the other is above 730 nm (Uludağ et al., 2004). However, it has to be noted

that these studies have used experimental adult data and theoretical models based on adults' rather than infants' head geometries. In functional studies of infants, lower wavelengths of 770 (e.g. Miguel et al., 2017) and 780 nm (Lloyd Fox et al., 2010) have both been successfully employed.

It is important to highlight that two different CW systems have been used to collect data presented in this thesis. Data for Experiments 1 and 2 have been collected using the UCL NIRS system (NTS2; Everdell et al., 2005) which uses 770 and 850 nm. Data for Experiments 3 and 4 have been collected using the NIRS system ETG-7000 (Hitachi, Tokyo, Japan) with wavelengths of 780 and 830 nm. Use of these two different wavelength pairs is supported by previous experimental and theoretical work based on adults and on work with infants.

One limiting factor of the CW method is that it cannot directly measure the non-linear trajectory of light (due to scattering phenomena) in biological tissue. Therefore, it cannot measure absolute concentration values HbO_2 and HHb and all measures are of changes in concentration.

Data acquisition and data analysis are specific to each of the experimental chapters that used fNIRS (Chapters 3 and 4) so details regarding the system, software, headgear and analysis steps can be found within the experimental chapters.

2.3 Eye-tracking

2.3.1 Introductory remarks

The main challenges that behavioural research with young infants pose are the impossibility to use verbal instructions and to expect complex behavioural responses. The introduction of looking time paradigms allowed researchers to overcome both. The pioneer of looking times studies was Robert Fantz who in the late 1950s introduced the 'preferential looking paradigm' (R. Fantz, 1958). This method exploits the fact that infants have a natural tendency to look at novel and conspicuous visual stimuli (Fantz, 1964, 1963, 1956) and measures how long infant's gaze is directed to one or more stimuli presented together. A remarkable amount of current knowledge on different aspects of infants' development, both perceptual and cognitive, relies upon the use of looking time measures. In a preferential looking paradigm, the infant is presented with a pair of stimuli and looking times to each of the two stimuli is measured and compared. If the infant looks for longer at one stimulus over the other it is inferred that he/she can discriminate between the two and that there is a preference for the stimulus that was looked at for longer. Another widely used paradigm that measured looking behaviour is that of 'habituation', where infants are repeatedly presented with the same stimulus until the time spent looking at it decreases by a predefined amount (they 'habituate'), at which point a novel stimulus is presented. If an increase in looking times to this new stimulus is recorded, then it can be inferred that infants can discriminate between the two stimuli.

Before the introduction of eye-trackers looking times were coded off-line, frame-by-frame, from video recordings. With offline coding finer-grained analysis were not possible because of the low spatial resolution of human coding (e.g., Aslin & McMurray, 2004). The advent of eye tracking introduced the possibility to exactly measure looking patterns allowing the accurate localization of the participant's gaze (with horizontal and vertical gaze coordinates) on a visual stimulus (using offline coding determining vertical gaze position is much more difficult than horizontal gaze position). Eye trackers record eye movements with a sampling rate ranging from 50 to 500 Hz thus

offering a higher temporal resolution compared to manual video coding (sampling rate of 30 Hz).

The benefits of eye tracking are not limited to highly spatially and temporally accurate estimation of gaze location. Gaze data obtained by the eye tracker can be accessed and analyzed in real time, a possibility that led to the implementation of gaze-contingent tasks. In such tasks changes in the displayed stimulus occur depending on where (or for how long) the viewer is looking, so it is the viewer who controls the progression of the experiment.

In the current thesis, eye-tracking was used mainly to measure latencies of disengagement from a central stimulus when a new stimulus appeared peripherally. Gaze contingent tracking was employed.

2.3.2 Eye-tracking technology

There are several techniques used to track the movement of the eyes but the most commonly used one is video-based pupil centre corneal reflection (PCCR). Systems that use this technique typically consist of an infrared camera, light-emitting diodes (LEDs, usually embedded in the camera) and image processing software to identify the features of the eye used for tracking. Infrared light is directed at the centre of the viewer's eyes (the pupil), causing strong reflections (a bright glint) in the cornea –also known as the *first Purkinje image*- that remains relatively stationary as the eye moves. It is this glint and its distance relative to the pupil that is used to estimate exact gaze position on the screen thanks to image processing algorithms implemented in the eye tracker hardware. It is necessary to use two points of reference on the eye to disambiguate between eye movements and head movements and to determine the position of the eye in space (also known as Point of Regard, POR). The positional difference between the corneal reflection and the pupil depends on eye rotation around vertical and horizontal axes (i.e., the eye ball rotation conditions the pupil position whereas the corneal reflection is relatively stable), but is not affected by small head movements (Duchowski, 2017). The outputs of tracking are the (x, y) coordinates of the viewer's gaze on the screen.

The relationship between corneal reflections and pupil depends on the characteristics of the viewer's eyes and on the position of the eyes relative to

the eye-tracker camera. Therefore, a calibration procedure prior to the recording is necessary in order to reliably determine the viewer's gaze position.

2.3.4 Data Acquisition

2.3.4.2 Apparatus

All the eye-tracking data in this thesis were collected using a remote Tobii TX300 eye-tracker (Tobii Technology AB, Danderyd, Sweden). The eye-tracking unit comprised a near-infrared light source and a camera with image sensors and was equipped with image processing firmware provided by Tobii Technology. The binocular gaze data were recorded with the sampling frequency of 120Hz.

Tobii TX300 tolerates large head movements allowing the infants the freedom to move during the stimuli presentation[16]. Specifically, the freedom of head movement at 65 cm was within 37 x 17 cm (width x height), and the maximum head-movement speed that could still lead to successful tracking was 50 cm/sec. The eye-tracker could recover gaze positions within 10-165 ms. Provided that the infant's head and body were relatively still, the accuracy of the current system ranges approximately from 0.5 to 1 degrees of visual angle (i.e., < 12 mm at a distance of 65 cm) across the entire screen. The eye tracker's latency was reported to be less than 10 ms (Tobii Technology AB, Danderyd, Sweden).

The system used for the present work (i.e., eye-tracking unit and the monitor) was mounted on a mechanical arm, which facilitated the adjustment of the eye-tracker position along both vertical and horizontal axes to quickly find the participant's gaze during the set-up period.

The visual stimuli were presented on a 23-inch thin-film transistor (TFT) liquid crystal display (LCD) monitor (attached to the eye-tracker unit), with the

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[16]Not all eye-trackers allow freedom of movement. Indeed, some spatially fixed or static eye-trackers require that participants keep their head still and often use forehead and/or a chin rest during the testing session. In contrast, *remote* eye-trackers (as the one employed for this thesis) allow for head movement within a certain area in front of the tracker also known as the *head box* (Holmqvist et al., 2011).

resolution set to 1024 x 768 pixels. The sound stimuli were played through external speakers placed symmetrically at the sides of the screen.

The participants were monitored and recorded through a video camera embedded in the upper part of the Tobii monitor by using the ScreenFlow screen-casting software.

2.3.4.3 Software

For the purposes of data acquisition (i.e., displaying stimuli, recording eye movements, and exporting data) we employed a MATLAB stimulus presentation framework developed by Dr. Luke Mason (https://sites.google.com/site/taskenginedoc/) which I modified to meet the needs of the current experiments. The data points were collected every 8.3 milliseconds. For each data point, a number of eye-tracker outputs were recorded (please see User Manual Tobii Technology, 2012). The onsets of the events of interest were marked by event markers generated by the stimuli presentation scripts.

The gaze contingency was implemented in the scripts by treating the gaze coordinates as mouse coordinates, therefore allowing the infant to perform actions such as initiating the next experimental trial by looking at appropriate items.

2.3.4.4 Calibration

Calibration procedures are of great importance since the validity of the recorded eye-tracking data depends upon accurate calibration. The main purpose of calibration is to adapt the parameters for the calculation of gaze direction to the participant's eye and to the peculiarities of the testing session (e.g. luminance of the room). Calibration needs to be performed for each participant because of differences in the eye features (e.g., the eyeball radius) and in the exact positioning in front of the eye tracker. Therefore, the correct interpretation of the images captured by the eye tracker camera relies on mapping the signals provided during the calibration onto the stimulus field. During calibration, the information about the participant's gaze points is

recorded and compared to a set of spatial locations on the stimulus monitor that are represented by the geometric centres of the calibration stimuli.

In this thesis, I employed the 5-point calibration sequence commonly used with infants. Infants' gaze was cued to five spatially confined screen locations (i.e., the four corners and the center of the screen) using colorful spinning balls. For most participants, successful calibration was achieved in less than 1 minute. In cases of unsuccessful calibration due to poor gaze tracking or to the infants not attending to the screen, the procedure was repeated.

The room light levels were constant throughout the experiment to prevent tracking errors resulting from changes in pupil size due to changes in lighting.

2.3.4.1 Overview of the testing procedure and experimental set-up

During the eye-tracking experiment (Experiment 5, Chapter 5) infants were seated in a Bumbo seat (secured to a chair) at approximately 65 cm away from the display and the eye tracker, with their caregiver sitting behind them. An attractive cartoon (*Waybuloo*) was played on the screen to attract the infants' attention to the screen so that the eyes could be positioned in the range of the eye tracker. The researcher would tilt the screen or adjust the participant's position until a good signal was reliably picked up from the eye tracker, at which point a 5-point calibration routine took place to ensure the spatial accuracy of the subsequently collected data. The calibration procedure was repeated if at least 4 out of 5 points were not well calibrated.

Following successful calibration, the researcher started the experiment which consisted in a modified version of the gap-overlap task (Elsabbagh et al., 2013). I used gaze-contingent presentations so the infants controlled the progression of the experiment, therefore the speed of presentation was individually adjusted. Furthermore, this provided the experimenter with an on-line feedback on the tracking quality. Without reliable gaze, contingency computations would not be possible and the stimulus presentation would not progress, thus an undisrupted presentation would indicate successful tracking.

Details regarding the task used, the stimuli and the analysis of reactions times can be found in Chapter 5, sections 5.22 and 5.2.4.

Chapter 3

Hand or spoon? Exploring cortical responses to affective touch in 5-month-old infants

Chapter 3 is based on the following article:

Pirazzoli, L., Lloyd Fox, S., Braukmann, R., Johnson, M. H., & Gliga, T. (2018). Hand or spoon? Exploring the neural basis of affective touch in 5-month-old infants. *Developmental Cognitive Neuroscience*.

3.1 Introduction

Infants are born into a social world and encounter a multitude of social cues across modalities: for example, the typical experience of a young infant involves frequently seeing their caregivers' faces and hearing their voices. Besides being mediated by social visual and auditory stimuli caregiver-infant interactions are also mediated by social touch. Despite striking parallels between human and animal work highlighted in the introduction, it is as yet un-known in what way social touch makes specific contributions towards our early development. For specificity to touch to occur, infants should be able to discriminate social touch from the multitude of tactile experiences they encounter, just as they discriminate other social signals such as faces and voices, from the variety of visual and auditory stimulation they are exposed to (e.g. Farroni et al., 2013; Grossmann et al., 2010; Lloyd-Fox et al., 2009) over the first year of life.

One way in which previous research has assessed the discrimination of social and non-social stimulation has been by observing the development of socially selective responses in the infant brain. Functional Near Infrared Spectroscopy (fNIRS) has been central to charting the development of specialization to a variety of social stimuli, from early infancy. By specialization I refer to the theoretical perspective of *Interactive Specialization* -IS (M. H. Johnson, 2001, 2011; M. H. Johnson & Munakata, 2005a). According to this view functional brain development in the cerebral cortex involves a process of specialization in which regions initially have very broadly tuned functions (and are activated by a wide range of different tasks/stimuli) that during development become increasingly finely tuned[17] (regions become specialised and their activity becomes restricted to a narrower set of task/stimuli) (M. H. Johnson, 2001, 2011; M. H. Johnson & Munakata, 2005b). Further, as cortical

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[17] According to IS the emergence of specialization within a region is partly determined by its patterns of connectivity to other regions, and their patterns of activity (Johnson, 2011).

regions become more specialized the degree of localization of the responses will also increase (activity elicited from a certain stimulus becomes more focal). The IS perspective is supported by evidence in several domains of social perception including the emergence of the social brain and social cognition (for reviews see (Grossmann & Johnson, 2007; Johnson et al., 2009).

Notably, fNIRS research that investigated the emergence of responses to social stimuli has indicated two areas as consistently engaged for the processing of social stimuli, across modalities: the superior-middle temporal and the inferior frontal cortices. The superior temporal sulcus (STS) runs along the temporal lobe and in adults the banks of this sulcus have been associated with processing faces, voices and biological motion (Deen et al., 2015). Recently, posterior areas around the sulcus have been described as a hub for multisensory integration (Beauchamp et al., 2008, 2004; Dahl et al., 2009), with the suggestion that the close proximity of the STS to all sensory cortices has led to its recruitment for processing highly multimodal social information. Indeed, the STS and the inferior frontal cortex show early specialization for social stimulation, across modalities.

3.1.1 STS and social information processing

In the visual modality, the posterior STS-temporoparietal junction area (pSTS-TPJ: includes the posterior middle and superior temporal gyri, STS and TPJ) already shows social selectivity in newborn infants (Farroni et al., 2013) and selective responses to a wide range of social visual stimuli (i.e. eye gaze shifts, "Peek-a-boo", static faces) are consistently reported in this area during early development (Biondi et al., 2016; Grossmann et al., 2008; Lloyd-Fox et al., 2011, 2009; Otsuka et al., 2007). Specialization to visual social stimuli has been found also in the frontal lobe. Indeed, some of these studies found activation in the inferior frontal gyrus (IFG) (Lloyd-Fox et al., 2009, 2011) and in the prefrontal cortex (Grossmann et al., 2008). In addition to these, other works reported prefrontal activation in response to viewing a smiling mother (Minagawa-Kawai et al., 2009) and to live interactions with direct gaze (Urakawa et al., 2015).

In the auditory domain, while social selectivity has also been reported, STS may specialize later in development. Interestingly, the pSTS-TPJ area

exhibits non-vocal selective responses during the first few months of life, responding to water, bells, rattles. Greater responses to non-voice sounds in this area have been reported in infants from 0 to 8 months (Grossman et al., 2010; Lloyd-Fox et al., 2016). Confirmation from fMRI with 3 to 7-month-old infants shows both voice selective (anterior middle and superior temporal gyri) and non-voice selective responses (posterior superior temporal gyrus) (Blasi et al., 2011). Selectivity to human vocal sounds has been reported to emerge between 4 and 7 months of age over the more anterior portion of the STS region (an area covering anterior STS and STG-MTG) (Blasi et al., 2011; Grossman et al., 2010; Lloyd-Fox et al., 2011, 2013, 2016), in line with the areas of vocal selectivity seen in adults (Belin, Zatorre, Lafaille, Ahad, & Pike, 2000)

Another study contrasted responses to human speech versus human non-speech vocal sounds (both social stimuli) and found responses to both speech and non-speech sounds over the posterior superior, middle and inferior temporal gyri in 1 to 4-month-old infants. Responses to speech were larger than those to non-speech, and interestingly responses to non-speech decrease with age, resulting in increased specialization for speech (Shultz, Vouloumanos, Bennett, & Pelphrey, 2014). Speech was also found to activate the IFG. Taken together these findings suggest that social selectivity in the temporal cortex seems to emerge to speech before that to non-speech sounds.
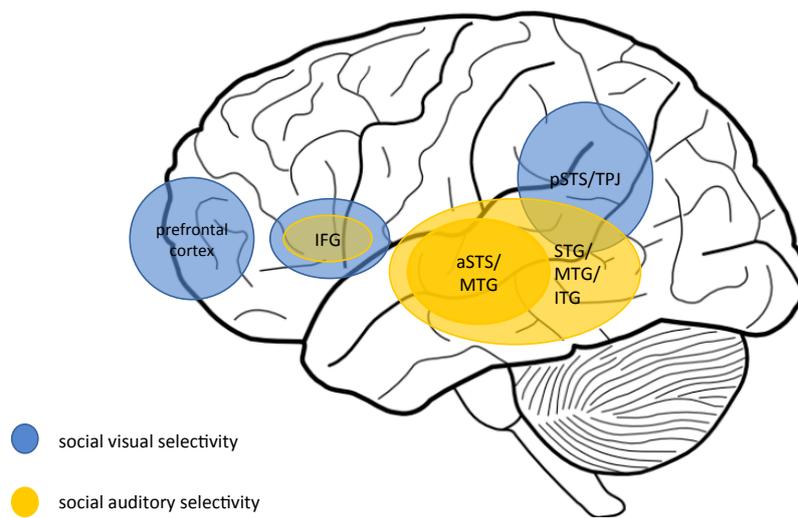
Figure 3.1 Depiction of cortical areas involved in visual (blue) and auditory (yellow) social processing in infancy. Social selectivity to visual stimuli has been reported in pSTS/TPJ, the IFG and the prefrontal cortex. Selectivity to vocal sounds has been reported in aSTS/MTG. Selectivity to speech sounds has been reported over the superior, middle and inferior temporal gyrus (STG/MTG/ITG) and in portions of the IFG.

Despite some consistency in social responsivity across modalities in recent research, this review highlights obvious differences in developmental trajectories of cortical specialization. pSTS-TPJ selective responses to *visual* social stimuli emerge shortly after birth. In contrast, adult-like selectivity to the *human voice* develops over the first months of life in anterior parts of the STS region, close to auditory sensory cortices. Selectivity to *human speech* seems to develop earlier over a broader area of the temporal cortex that encompasses the superior, middle and inferior temporal gyri. Furthermore, regions around the pSTS show selective activation to non-human sounds over this same period before subsiding, to be replaced by more general responses to auditory stimulation at later ages. Differences in specialization could be due to familiarity with the stimuli. Non-human sounds might represent a novel stimulus for a young infant, eliciting a larger response than the more familiar vocal sounds. As experience of vocal and non-vocal sounds accumulates, the large responses to non-social stimuli start subsiding and the pSTS becomes only selective to social stimuli, including speech. We cannot be sure as to why an area involved in social processing shows preference for non-social stimulation. There are only a handful of studies that measured responses to social vs. non-

social auditory stimuli and these often employed different contrasts, thus not warranting firm conclusions. There is certainly a necessity to further understand changes in processing of social stimuli across development, and studying how social selectivity in another sensory modality (tactile) develops may help in this direction. As evidence from different modalities and age-groups accumulates, it will be possible to shed light on how specialization in the social brain develops differentially to support social processing in different sensory modalities.

3.1.2 STS and tactile processing

In contrast to the abundant evidence from the visual and auditory domains, only a few studies to date have investigated social selectivity in the tactile domain. As it has been described in Chapter 1, these have been mainly driven by the discovery of CT afferents in the hairy skin of humans. Given their functional properties (they are velocity- and temperature tuned to caress-like touch) it was proposed that these afferents encode affective properties of social touch. This led to the publication of several studies over the last five years that have investigated responses to affective (defined as CT-optimal touch) versus non-affective touch in adults (defined as tactile stimulation suboptimal for eliciting CT activity). These studies found consistent patterns of activation in IFG and pSTS (Bennett et al., 2014; Gordon et al., 2013; Voos et al., 2013), but see (Davidovic et al., 2016).

However, studies that have investigated affective touch processing in infancy are few, and results are inconsistent (Saito, 2009; Kida and Shinohara, 2013; Miguel et al., 2017; Jönsson et al., 2017; Tuulari et al., 2017). In two of these works (Saito, 2009; Kida and Shinohara, 2013) affective touch is not defined based on a CT model; instead of manipulating speed, temperature or skin site (hairy vs. galbrous) these works contrasted different textures (pleasant vs. neutral). Yet, I have still included these studies here as while the contrast was not aimed at eliciting differential CT afferents activity, the pleasant stimulation conditions (gentle stroking with a soft object) can be considered CT-optimal. The pattern of responses observed from these infancy studies (reviewed in detail in the introduction) using different textured stimuli,

or tactile stimuli applied at different speeds, has not illuminated a clear developmental pathway of specialization. This stands in contrast with fNIRS and fMRI studies of affective touch in adulthood. Interestingly, a study that looked at the development of these responses from childhood to adulthood found that a region of the middle temporal gyrus (MTG) extending into the pSTS was activated by affective touch as early as 5 years of age, while frontal areas only consistently activated in adulthood (Bjornsdotter et al., 2014). This would suggest that responses to social touch may develop differentially across different social brain regions.

Given the limited evidence from early development, I set out to clarify the involvement of STS and IFG in social selectivity to touch during early infancy. I aimed to build on previous work in two ways. First, in some of the previous studies (Saito, 2009; Kida & Shinohara, 2013) measurements were restricted to a confined region of the anterior prefrontal cortex, or to only the right (Miguel et al., 2017) or the left STS region (Jönsson et al., 2018), therefore inferior frontal and posterior-temporal responses in infants have not been extensively investigated. Second, tactile stimulation may not have been optimal, for example in Kida & Shinohara, (2013) stimulation was delivered to the palm of the hand, a region that lacks CT afferents (Johansson & Vallbo, 1979; Johnson et al., 2000, Vallbo et al., 1999; Wessberg et al., 2003; Löken et al., 2009). Third, the presentation of touch during these studies was usually concurrent with the infant being embraced or held by their caregiver, with the caregiver and the experimenter administering the touch stimulus within their field of view (i.e. Jönsson et al., 2017). Therefore, responses may have been altered due to the context of the stimulus, as we cannot exclude that infants processed the stimulation as originating from their caregiver. It is indeed possible that infants in Jönsson et al. (2017) thought that they were being stroked by their mother. Therefore, in the present study, I delivered stimuli to the upper arm and recorded responses from the inferior frontal and the posterior temporal cortex over both hemispheres. Since I was interested in characterizing the response to the affective touch in isolation from other social cues, I ensured that the infants did not see who was performing the stimulation. Furthermore, infants were placed in an infant carrier, on their parents' lap and parents were asked to refrain from touching the infant during the study.

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To study social touch, I chose CT-afferents mediated affective touch as the physical properties of this stimulus have been clearly defined (providing a stimulus that can reliably be adopted across studies) and the neural underpinnings of this stimulus have been charted (and robustly replicated) in adults.

I contrasted responses to affective and non-affective touch, compared to a no tactile stimulation baseline. The affective touch was delivered by a human hand at CT-targeted velocity. I contrasted this with a non-affective stimulus, which was performed at the same speed but with a metal spoon; this was designed to differ from the social affective touch in temperature, the spoon being at room temperature. Recent research had shown that CT firing and pleasantness ratings decreased when tactile stimulation was applied at 18°C (room temperature) compared to human skin temperature (32°C; Ackerley et al., 2014). It was suggested that temperature may be one of the key properties of human touch, ensuring thermoregulation early in life when infants themselves poorly regulate their body temperature (India Morrison, 2016b). In this way, I sought to tease apart the relative contribution that this factor may have on the social affective response previously observed by manipulating the form of touch in other dimensions. Therefore, I hypothesised that affective touch as delivered through stroking with the hand would lead to increased activation in the pSTS-TPJ region and in IFG, relative to the control stimulation. I chose to investigate these responses at a similar age (5-6 months) to when previous research has shown socially selective responses in the visual and auditory domains. Stronger activation for affective versus non-affective touch in these areas, would allow to infer that cortical specialization to the affective components of touch has already started to emerge by early infancy.

3.2 Methods

3.2.1 Participants

Twenty-one five-month-old infants participated in this study (8 female, mean age = 160.19 days, SD = 13.91). A further 8 infants participated but were excluded from the study owing to an insufficient number of valid trials based on behavioural coding (4) or a high level of rejected data due to motion artifact (4).

All infants were born full term (37–42 weeks' gestation) and with normal birth weight (>2500 g). This attrition rate is within the standard range for infant fNIRS studies (see review by Lloyd-Fox et al., 2010). All parents gave written informed consent before the study and the ethics committee at Birkbeck, University of London, approved the study design.

3.2.2 Stimuli and design

Each stimulus trial was 10 seconds long (Figure 3.2a). The affective touch condition consisted of a gentle stroke, in the velocity range of 3-10 cm/s, performed by the experimenter on the baby's upper arm, with repeated stroking applied horizontally from the inner arm across to the outer arm. To time the presentation of the stimuli, the experimenter listened to audio cues played in headphones which indicated the beginning and the end of each trial. Each stimulus trial consisted on average of 5 strokes (1 stroke every two seconds), given that the upper arm of the infants in our sample had a length of 10cm and I administered a stroke velocity which allowed us to cover this length of skin in 2 seconds. Since infants' unpredictable movements can induce alterations to this speed (if they move their arm during stimulation) the stroke could vary in speed. Offline coding confirmed that the range of 3-10cm/s (which is the range in which CT fibers are reported to fire optimally), was not exceeded for any participant as the maximum number of strokes in 10 seconds was never larger than 6. If the experimenter was halfway through a stroke when the end of the trial was signaled she would complete it, which could add an additional one-two seconds to the duration of the stimulation. In the non-affective touch condition, the arm was stroked by using the back of a spoon at the same speed. Following each 10 second trial there was a period of no-touch baseline which lasted 10 seconds. Half of the participants received stimulation on the right arm, the other half on the left arm. The order of presentation of the stimuli (hand/spoon) was counterbalanced across participants, with half of the participants receiving the hand stimulation on the first trial, and half the spoon stimulation, with trials alternating in an ABAB sequence thereafter. During the procedure participants watched a colorful screensaver accompanied by music, to avoid them orienting to the tactile stimulation.
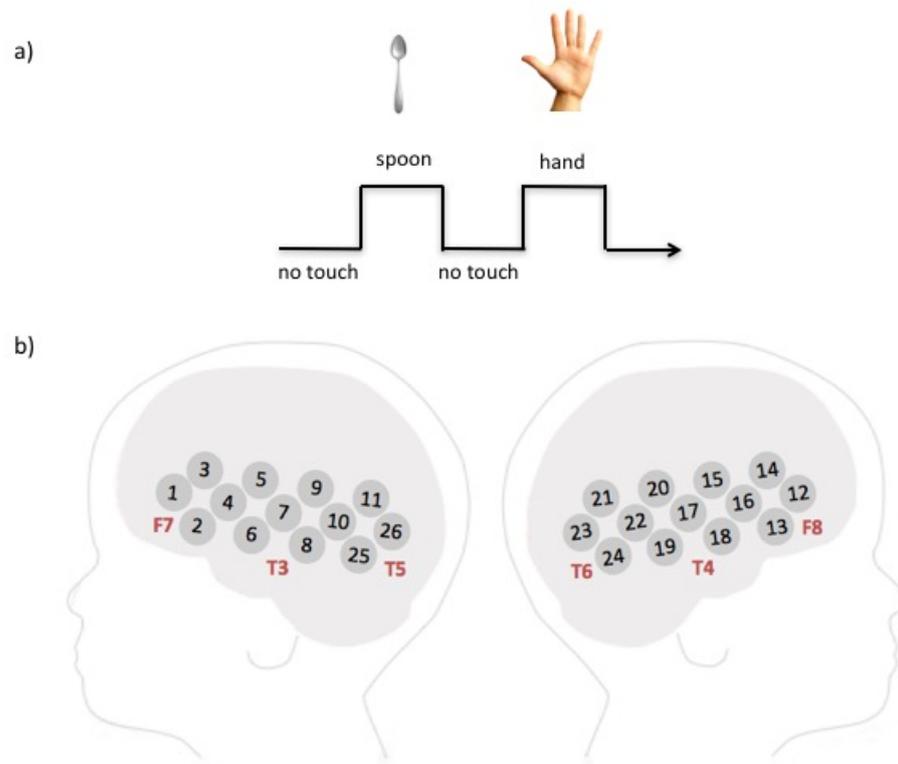
Figure 3.2 a) Experimental design: the stroking was performed using a spoon or a hand; experimental and baseline periods were 10 seconds long. b) A schematic showing the location of the channels relative to the 10-20 coordinates (in red)

3.2.3 Apparatus

Infants wore custom-built CBCD NIRS headgear (http://cbcd.bbk.ac.uk/node/165) consisting of two source-detector arrays containing a total of 26 channels (source-detector separations: 20 mm). The arrays were placed over both hemispheres and covered the inferior frontal - temporal lobes (see Figure 3.2b). Data was collected with the UCL NIRS system (NTS2; Everdell et al., 2005). This system used 2 continuous wavelengths of source light at 770 and 850 nm. Before the infants began the study, measurements of their head circumference, ear to ear lateral semi circumference, and nasion to inion were taken, and the location of the channels and arrays relative to these anatomical landmarks were recorded (Lloyd-Fox et

al., 2014). Measurements from this group of infants showed that the average head circumference was 43.16 cm (SD = 1.81).

3.2.4 Procedure

The infants were held on their parent's lap, secured in a baby carrier, and facing outwards towards a 117-cm plasma screen. I chose to use the baby carrier in order to reduce the amount of tactile contact between the parent and the baby, thus isolating the touch delivered by the experimenter. The parent was asked to place their hands on the carrier rather than their infant, and refrain from interacting during the stimuli presentation unless the infant became fussy or sought their attention. The experimenter stood behind the parent and the infant, and delivered the tactile stimulations on the baby's arm, being careful to remain out of the baby's sight. Events (trial onset and offset) were marked on-line by a second experimenter observing the first experimenter on a computer monitor. The experiment ended when the infants became fussy. Each session was recorded using a video camera placed just below the screen, and infant behaviour was coded offline.

3.2.5 Data processing and analysis

The fNIRS system measured changes in the amount of light that was emitted from the sources, and detected by neighbouring detectors. These changes in light attenuation were used to calculate changes in oxy– (HbO_2) and deoxy–haemoglobin (HHb) chromophore concentration (µMol) which are haemodynamic indicators of neural activity (Obrig & Villringer, 2003). Prior to conversion to concentration data, the attenuation measurements for each infant were analysed and channels were rejected from further analysis based on the quality of the intensity signals, using artifact detection algorithms (Lloyd-Fox et al., 2010; Lloyd-Fox et al., 2009). In line with previous work, channels were excluded if the coefficient of variation of the attenuation exceeded 10% or if the normalized power was larger than 50% with respect to the total power (Lloyd-Fox et al., 2009). The attenuation signal was low-pass filtered using a cut-off frequency of 1.7Hz. Following this, the data was segmented into blocks of 24 seconds of data consisting of 4 seconds of the baseline prior to onset of the

tactile stimulation, 10 seconds of tactile stimulation, plus the following 10 seconds' baseline. Each block of attenuation data was de-trended with a linear fit between the average of the first and the average of the last 4 seconds to remove drifts in the signal. The attenuation data was then converted into changes in concentration (µMol) in HbO_2 and HHb using the modified Beer–Lambert law (Delpy et al., 1988) and assuming a differential path length factor for infancy (5.13; based on Duncan et al., 1995). Following this, trials were assessed both with motion detection algorithms and offline coding of infant behaviour. Trials were firstly removed if during the 4 seconds' baseline prior to the onset of the stimulus trial there were concentration changes greater than +/- 3 µMol, and if during the stimulus trial itself changes exceeded +/- 5 µMol (these thresholds were set at different levels to take into account changes in haemoglobin levels caused by activation during stimulation). In addition, experimental trials were removed following offline coding of infant behaviour. A trial was removed if: a) the infant moved to a degree that it prevented the experimenter from completing a sufficient number of strokes b) the infant turned to look either at the parent or the experimenter c) the parent interfered by either talking to or touching the infant. Not looking at the screen did not constitute a criterion for exclusion. Across the whole group, I rejected individual data on only three occasions because of an infant's movement, and on one occasion because of parent interference. Further details of the number of presented and valid trials, for those infants included in analysis, can be found in Table 3.1. For each infant, a channel was included in the statistical analysis if it contained at least three valid artifact-free trials per condition. It follows that at the group level not all infants contributed data to each channel. In addition, to include an infant in the final dataset a minimum of two thirds of the channels within the arrays were required to have valid data (i.e. not rejected during artifact detection algorithms).

Valid trials for each experimental condition *(affective touch, non-affective touch)* were averaged together for each infant, and a time course of the mean change in HbO_2 and HHb concentration changes was compiled for each channel. A baseline of 1 second of data pre-stimulus onset was subtracted from the signal. Two time windows were selected for analysis, between 1 and 5 seconds and between 5 and 9 seconds post-stimulus onset. These periods of time were

selected to include the range of maximum concentration changes observed across infants for HbO_2 and HHb. Either a significant increase in HbO_2 concentration from baseline or a significant decrease in HHb is commonly accepted as an indicator of cortical activation in infant work (Lloyd-Fox et al., 2010).

3.2.5.1 Preliminary analyses

A preliminary channel-by-channel analysis was run to identify those channels that responded to touch, irrespective of condition. This was achieved by comparing the response to the experimental trials to the pre-stimulus signal across all infants, using the valid data for each channel. Statistical comparisons (two tailed t-tests) were performed, to compare the maximum signal change during the specified experimental trial time windows, with the averaged pre-stimulus signal (4 seconds pre-onset). To account for errors due to multiple comparisons, p-values were corrected using a MATLAB false discovery rate (FDR) function (Benajmini & Hochberg, 1995).

3.2.5.2 Analysis plan

Channels that survived FDR corrections together with the homologous channel in the opposite hemisphere were analysed using linear mixed models (LMM) to account for side of stimulation and hemispheric effects. For each pair of channels, a linear mixed model was run, with hemisphere (right, left), stimulus (hand, spoon) and time-window (1-5s, 5-9s) as repeated measures factors, and side of stimulation (right, left) as between-subjects factor. I chose to use the LMM approach because of missing values occurring due to subjects not contributing data to some of the channels. LMMs use maximum likelihood estimation to handle missing values as compared to standard factorial analysis, where any subject not contributing data to all channels would be excluded from the analysis.

	Experiment 1
n	21
age (days)	160.19(13.91)
female/male	8:13
head circumference (cm)	43.16(1.81)
number of trials completed	10.87 (1.90)
valid trials	10.61 (2.01)
valid trials in affective touch condition	5.23 (1.09)
valid trials in non-affective touch condition	5.38 (0.97)
number of rejected channels per infant	0

Table 3.1 Participants' information. The number of valid trials refers to the number of trials included in the analysis after off-line coding of the infant's behavior during the study. The first number refers to the mean value across the group and the bracketed number refers to the standard deviation.

3.3 Results

In an initial channel-by-channel analysis of the fNIRS data, t-tests compared the averaged hemodynamic peak changes in HbO_2 and HHb (during the time windows of activation described in the methods) evoked by the hand and spoon conditions to a baseline consisting of the 4 seconds preceding the stimulus, in which no touch was applied. Here we report only the channels that showed significant increases in HbO_2. The hand condition revealed significant increases in HbO_2 in three channels (ch. 3, 9, 10) in the left hemisphere, in the first time-window post stimulus onset (1 to 5 s), while the spoon condition revealed significant increases in HbO_2 in four channels (ch. 5, 9, 20, 14) bilaterally in the second time-window post stimulus onset (5 to 9 s) (see Figure 3.3). All channels reported here survived FDR corrections (see Table 3.2 for a complete list of channels). It is worth noting that whilst no channels survived FDR corrections for the hand condition in the second time-window (from 5 to 9 s), six channels showed an uncorrected significant ($p < 0.05$) increase in response to the hand relative to baseline, including channel 3 and 9 (see Table 3.2).

I used the standardized scalp surface map of fNIRS channel coordinates within the frontal and temporal lobes specific to 4–7 months old infants (Lloyd-

Fox et al., 2014), to identify the most likely cortical regions generating the observed effects in the FDR corrected channels. Channels in which hand elicited a response (versus baseline) are positioned approximately over regions of the left IFG (ch.3) and left pSTS-TPJ (ch. 9, 10), while spoon touch elicited a bilateral response overlaying regions of the right IFG (ch. 14), bilateral pSTS-TPJ (ch. 9, 20) and left precentral gyrus (ch. 5).

To investigate hemispheric differences between the two conditions, each of the channels showing a significant HbO_2 response to either stimuli was paired with the homologous channel in the opposite hemisphere, resulting in four pairs: pair1(ch. 14 and 3), pair2 (ch. 20 and 9), pair3(ch. 15 and 5), pair4 (ch. 22 and 10), and analysed using LMMs.

For pair1 (IFG) I found a main effect of hemisphere ($F(1, 20.951)=4.926$, $p=0.037$), with greater activation in the left (M=.640, SE= .137) compared to the right hemisphere (M=.360, SE=.102), and a significant interaction between hemisphere and stimulus ($F(1, 21.182)=5.199$, p=.033). Post-hoc t-test revealed that the hand elicited a response in the left but not in the right hemisphere (t=2.068, p=.053) while there were no hemispheric differences for the response to the spoon, (t=.383, p=.706) (for the time courses of these channels see Figure 3.4, left panel). Neither of the other two factors included in the analysis, time-window and side of stimulation, did yield to significant effects (time-window: $F(1, 74.797)=.001$, p=.889; side of stimulation: $F(1,24.498)=.016$, p=.91).

For pair2 (pSTS-TPJ), I found a significant interaction between hemisphere and stimulus ($F(1, 34.994)=6.639$, p=.014) with both stimuli eliciting responses in both hemispheres but the hand activated the right hemisphere to a lesser degree than the left. However, this hemispheric difference, which can be observed in Figure 3.4 (right panel), did not reach statistical significance (t=1.46, p=.160). Also in this analysis, neither time-window ($F(1, 52.270)=1.242$, p=.270) nor side of stimulation ($F(1, 20.901)=.471$, p=.5) yielded to significant effects.

Analysis of the remaining two pairs yielded no main effects nor significant interactions (p>.2).
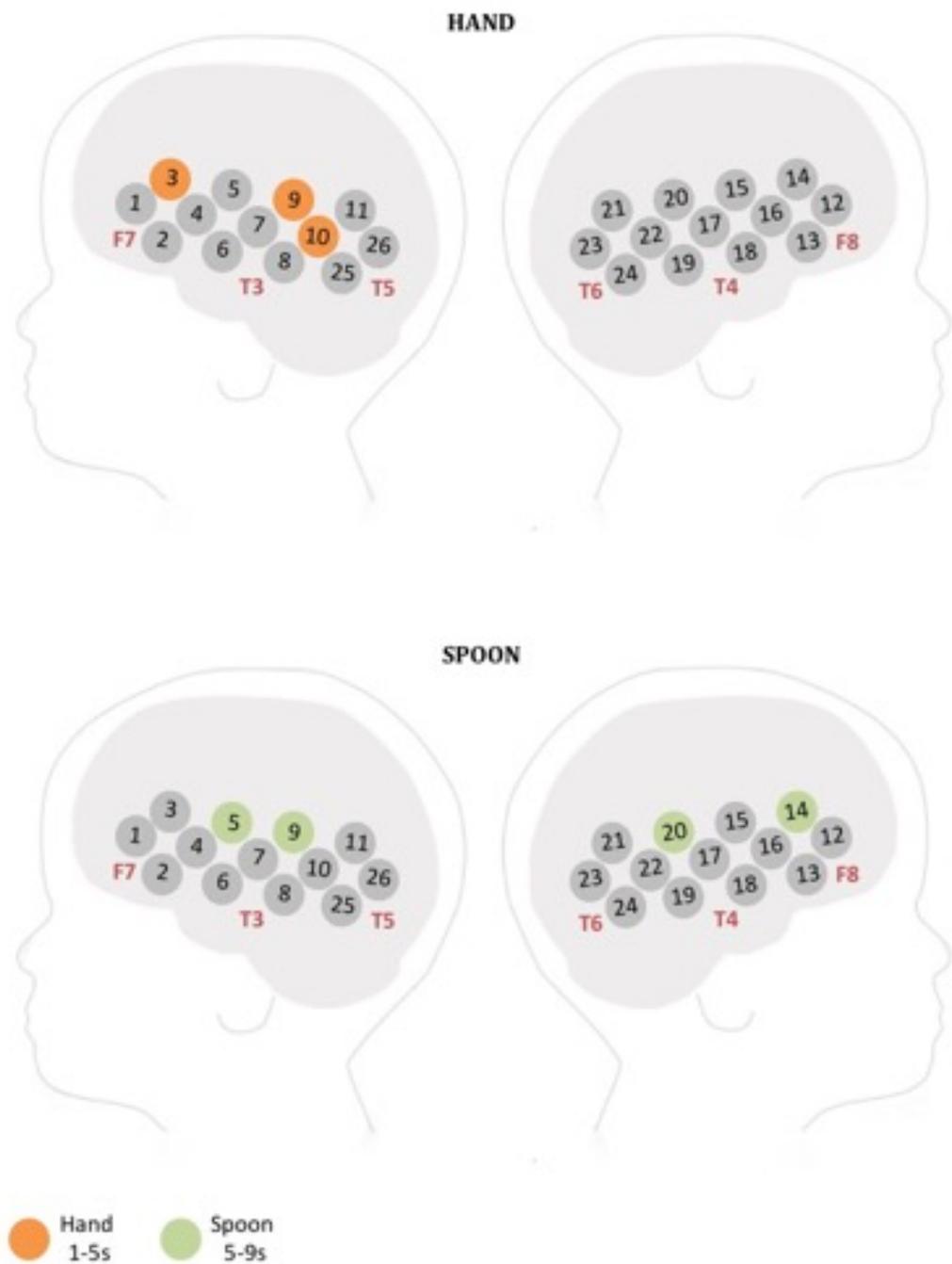
Figure 3.3 A schematic view of the NIRS arrays showing HbO$_2$ responses to the hand (top panel) and to the spoon (bottom panel). Channels marked in bright orange revealed a significant response in the 1-5s time-window to the hand versus baseline. Channels marked in pale green revealed a significant response in the 5-9s time-window to the spoon versus baseline
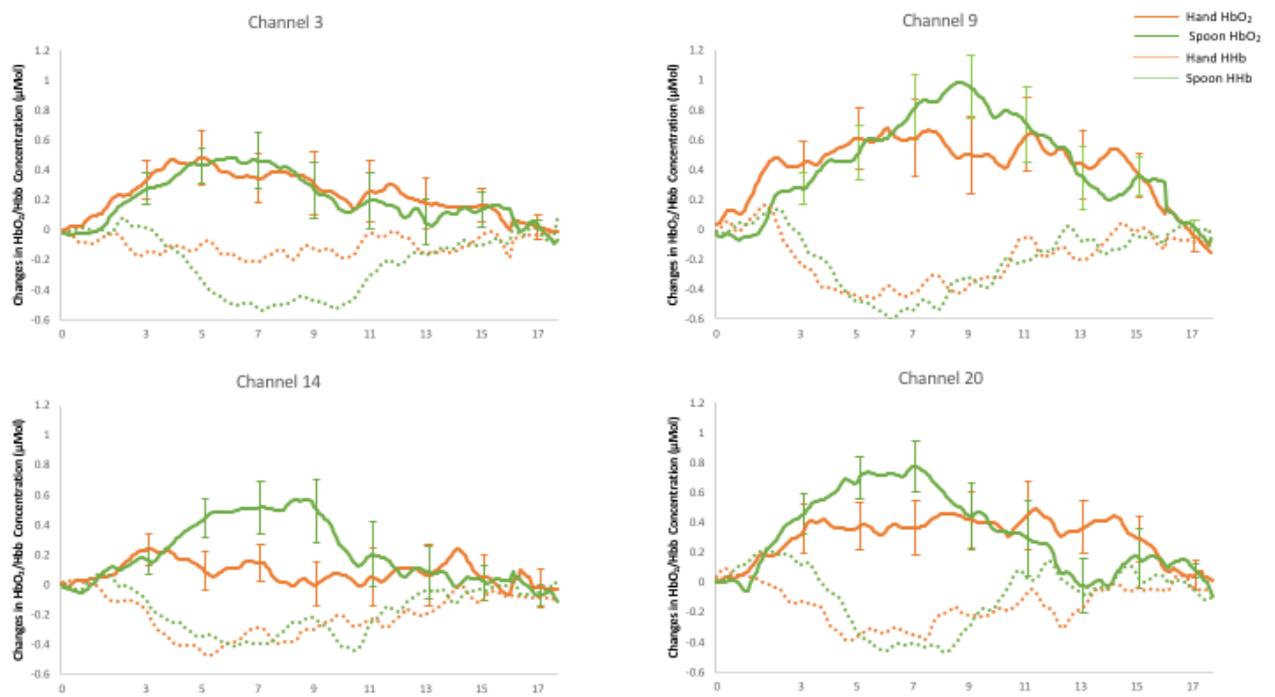
Figure 3.4 Grand averages of haemodynamic time courses within channels that showed significant responses and are centered within two key areas known to respond to affective touch: IFG (Ch. 3 left and Ch. 14 right) and pSTS-TPJ (Ch. 9 left and Ch. 20 right). Error bars represent standard error

	Hand > Baseline							Spoon > Baseline					
Ch	HbO_2/ HHb	TW	t	p	df	d	Ch	HbO_2/ HHb	TW	t	p	df	d
1	HbO_2	1-5	2.45	0.025	18	0.53	3	HbO_2	1-5	2.13	0.048	18	0.46
3*	HbO_2	1-5	3.07	0.007	18	0.67	3	HbO_2	5-9	2.17	0.044	18	0.47
3	HbO_2	5-9	2.32	0.032	19	0.51	5	HbO_2	1-5	3.08	0.006	18	0.67
5	HbO_2	1-5	2.33	0.031	19	0.51	5*	HbO_2	5-9	3.74	0.002	18	0.82
5	HbO_2	5-9	3.53	0.002	19	0.77	9	HbO_2	1-5	2.74	0.013	20	0.60
9*	HbO_2	1-5	3.42	0.003	19	0.75	9*	HbO_2	5-9	4.09	0.001	20	0.89
9	HbO_2	5-9	2.67	0.015	19	0.58	11	HbO_2	5-9	2.69	0.014	19	0.59
10*	HbO_2	1-5	3.62	0.002	20	0.79	12	HbO_2	5-9	2.29	0.033	20	0.47
11	HbO_2	5-9	2.18	0.041	20	0.48	14	HbO_2	1-5	2.45	0.023	20	0.67
15	HbO_2	1-5	2.38	0.028	20	0.52	14*	HbO_2	5-9	3	0.007	20	0.82
16	HbO_2	5-9	2.09	0.05	20	0.46	15	HbO_2	5-9	2.38	0.027	20	0.60
19	HbO_2	5-9	2.09	0.049	20	0.46	20	HbO_2	1-5	2.83	0.01	20	0.89
20	HbO_2	1-5	2.74	0.013	20	0.60	20*	HbO_2	5-9	3.7	0.001	20	0.59
22	HbO_2	1-5	2.31	0.032	20	0.50	21	HbO_2	5-9	2.83	0.01	20	0.50
14	HHb	1-5	-2.4	0.026	20	-0.52	26	HbO_2	1-5	2.13	0.046	20	0.54
14	HHb	5-9	-3.3	0.004	20	-0.72	3*	HHb	5-9	-3.01	0.008	18	0.66
15	HHb	5-9	-2.65	0.016	20	-0.58	9	HHb	5-9	-2.33	0.03	20	0.52
21	HHb	5-9	-2.36	0.028	20	-0.52	10*	HHb	5-9	-4.07	0.001	20	0.62
							11	HHb	5-9	-2.26	0.036	19	0.81
							12	HHb	5-9	-2.4	0.026	20	0.62
							14*	HHb	5-9	-4.73	<0.001	20	0.46
							15*	HHb	5-9	-3.26	0.004	20	-0.66
							16*	HHb	5-9	-2.65	0.015	20	-0.51
							19	HHb	5-9	-2.23	0.038	20	-0.89
							21*	HHb	5-9	-3.09	0.006	20	-0.49
							22*	HHb	5-9	-2.92	0.008	20	-0.52
							23	HHb	5-9	-2.42	0.025	20	-1.03
							24*	HHb	5-9	-2.85	0.01	19	-0.58

Table 3.2 Significant activations from baseline in Hand Spoon conditions. * indicates that the response survived the false discovery rate (FDR) correction

3.4 Discussion

The aim of the present study was to investigate the development of responses to affective touch in regions of the frontal and temporal cortex in infancy: specifically, I aimed to investigate whether infants exhibited selective cortical responses to the processing of affective components of tactile stimulation by five months of age. Using fNIRS, I focussed on two regions of the cortex known to be selective to visual and auditory social stimuli in infancy - and that have been shown to activate in recent affective touch studies in infancy and adulthood (Gordon et al., 2013; Jönsson et al., 2017;Voos et al., 2013; for a recent meta-analysis see Morrison, 2016) - the inferior frontal and posterior superior temporal cortex.

Our choice of stimulus contrast was informed by research suggesting CT-fibers, present in human hairy skin, mediate the perception of affective touch. I hypothesised that the human hand (affective touch stimulus) will generate increased responses in regions of the pSTS-TPJ and IFG, compared to stroking with a metallic spoon, a stimulus with sub-optimal temperature (Ackerley et al., 2014). Contrary to this prediction, I found that both the hand and the spoon stimulation elicited a significant cortical response relative to baseline over these regions.

Exploratory analyses (channel-by-channel t-tests) revealed differences in the latency of the peak response, with only the response to the hand differing from baseline in the early time window; however this was not a significant factor in the main linear mixed model analyses. Rather, an interaction between hemisphere and stimulus was observed. The non-affective stimulus (spoon) elicited IFG and pSTS-TPJ responses bilaterally, while responses to the hand were left lateralized in the inferior frontal (minimal responses observed over right IFG) and posterior temporal regions (with a reduced response observed over right pSTS-TPJ). Note that this hemispheric difference was not a main driver of the results, as it was found to have borderline significance. This more localized response to the hand, if replicated, could indicate that at this age specialization and localization of cortical processing of affective touch are ongoing.

The only other two studies to date that measured posterior temporal cortex activation to affective and non-affective touch support our findings. In line with our results, they reported differential activation to affective versus non-affective touch in the left (Jönsson et al., 2018) but not in the right hemisphere (Miguel et al., 2017). However, it is hard to draw firm conclusions regarding lateralization from these findings as both studies restricted measurement to one hemisphere. Also, direct comparison of hand and spoon stimulation in our study, did not reveal statistically significance differences in either hemisphere. Thus, although some trend differences were observed between the two stimuli, they remain to be confirmed.

What could explain the differences between our findings and those of Jönsson and colleagues? One difference lies in the nature of the contrast investigated, as Jönsson and colleagues compared slow and fast velocity stroking. It is possible that while cortical specialization to touch velocity is already evident shortly after birth, sensitivity to human body temperature may take more time to develop. Texture, another critical aspect of affective touch, also shows protracted cortical specialization. Kida and Shinohara (2013) showed increased responses to pleasant touch, over the anterior prefrontal cortex in 10-month-olds, but not in 3 and 6 months-old infants.

Therefore, in light of findings from Jönsson et al. (2017), in the next set of studies presented in Chapter 4 I incorporated speed differences in our stimuli contrasts. One limitation of the present study is that while the metal spoon was certainly colder than the human body, temperature was not rigorously controlled. Thus, it is possible that the spoon warmed up during repeated stroking, making the difference between the two stimuli too subtle to elicit differential responses. Manipulating speed should lead to a clearer distinction compared to the one achieved manipulating temperature. The approach of facilitating the discrimination of the stimuli based on their physical properties is the option I chose to pursue given the initial goal of isolating responses to affective touch in absence of any contextual cues.

Chapter 4

Hand, toothbrush or brush? Exploring cortical responses to different stimuli contrast in 5 and 10-month-old infants

4.1 Introduction

Results from Experiment 1 (in Chapter 3) showed that both the hand and the spoon stimulation elicited a significant cortical response relative to baseline over a network of areas previously associated with processing visual social cues: the pSTS-TPJ and the IFG. The differences in activation between the two conditions were subtle. While this is compatible with lack of preferential responding to affective touch, it may also be that the particular contrast I used failed to reveal this specialization, by making both stimulations akin to affective touch. Sensitivity to human body temperature, the main contrasting dimension used in this experiment, may take more time to develop than other types of stimulus difference.

Therefore, in order to facilitate discrimination of the stimuli, I decided to increase the physical difference between the affective and the non-affective stimulus. I did so over two experiments (2 and 3). In Experiment 2, I maximized this difference by contrasting the stroke of a human hand (similar to the one used in Experiment 1) to the tactile stimulation delivered with an electric toothbrush. In this instance the non-affective stimulus differed from the affective one on 4 dimensions: speed of the stroke (fast speed suboptimal for CT fibers), presence of vibration motion ($\approx$ 200Hz vibration) which has been shown to poorly activate CT afferents (Olausson et al., 2002, Bessou et al., 1971), texture (rougher than the human hand) and temperature (room versus body temperature).

In Experiment 3[18] I tested the classical contrast adopted in adults' studies on affective touch: slow versus fast brush stroking. These stimuli were also similar to those employed in previous work with 2-month-old infants that revealed responses to affective touch in STS and in the insula (Jönsson et al., 2017). Despite conceptual similarities, there are obvious differences between the pairs of stimuli used across this new set of experiments. While four

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[18] At the same time while collecting data for Experiment 2, I was offered the opportunity to collaborate with a team at Keyo University (Tokyo, Japan) who were also measuring fNIRS responses to CT versus non CT- targeted touch in infancy and sought collaboration for data analysis and interpretation. Data collection was carried out at the Keyo infant lab by YM, YH and AK. Data analysis and interpretation was performed by LP.

dimensions differentiate hand from toothbrush stroking, only one dimension (speed) is manipulated in Experiment 3.

Experiment 2 uses skin-to-skin touch versus indirect human touch (through a toothbrush). Although the pressure exerted could be more inconsistent on a trial by trial level since two different methods of human administration are used, I nonetheless think that a human caress, if it bears the significance for development highlighted thus far, should be recognized regardless of naturally occurring variations in pressure. Instead, in Experiment 3 both stimuli are administered through the same tool (brush) which I believe is likely to lead to more consistent pressure. The advantage of measuring cortical responses with both these stimuli contrasts, even if across studies, is that it could reveal to what extent results obtained with brush stroking are comparable to responses elicited by realistic interpersonal affective touch, i.e. *more ecologically valid affective touch stimulus*. In adults, it was shown that stroking with a human hand compared to stroking with an inanimate object (velvet stick) elicited larger responses both in somatosensory regions and in the posterior insula (Kress et al., 2011). Indeed, especially for infants, that are exposed to a great amount of human touch during the first few months of life, using indirect human touch as 'affective touch' raises questions about ecological validity. *Do infants process controlled CT targeted touch delivered through a brush in the same way as a caress from their mother?*

Besides investigating the processing of affective touch at around 5 months of age, given that the current literature has an age gap between 7 months old infants (Miguel et al., 2017) and children (Bjorsndotter et al., 2014), I set out to measure responses to affective touch nearer to the end of the first year of life. Therefore, following Experiments 2 (5 month-old infants) and 3 (7 month-old infants), Experiment 4 extends the use of the same paradigm as Experiment 3, to the study of 10-month-old infants. Thus Experiment 4 builds on Experiment 2 and 3 to reveal any developmental changes across a five months age span.

Given that the stimuli contrasts across the three experiments are conceptually similar, while age sets apart Experiments 2 and 3 from 4, I put forward two hypotheses: (i) one stimulus related and (ii) one age related. For all three studies, (i) I hypothesized that if the similarity between stimuli is what

prevented/hindered a discrimination in Experiment 1, distancing further apart their physical properties should facilitate it. I therefore expected that affective touch as delivered through stroking with the hand or the brush would lead to increased activation in the pSTS-TPJ region and in IFG, relative to the control stimulation.

However, should the findings from Experiments 2 and 3 not support the original hypothesis (i) this might suggest that cortical specialization to affective touch (in the regions investigated) is still ongoing between 5 and 7 months of age. Experiment 4 will then clarify the role age plays in the development of these responses. Thus, if longer exposure to tactile stimuli is necessary for the cortical nodes of the social brain to tune to the affective properties of tactile stimuli, I hypothesize (ii) that a pattern of responses that resembles that seen in adults will emerge at 10 months.

4.2 Methods - Experiment 2

4.2.1 Participants

Twenty-one 4-6-month-old infants participated in this study (9 female, mean age=158.84 days, SD = 15.96). A further 21 infants participated but were excluded from the study owing to fussiness (n = 13), a high level of rejected data due to motion artifacts (n = 6), or interference of the parent talking to or touching the infant during the study (n=2). Attrition rate for Experiment 2 was much higher compared to the previous experiment (50% vs. 27.6%). This was caused by the fact that the length of baseline trials was 10s longer in Experiment 2 vs. Experiment 1. This change increased the overall duration of the experiment and completion of the minimum number of trials took longer. The longer duration coupled with the absence of social cues therefore increased the chances that the infant grew bored and fussed out prior to reaching the minimum number of trials valid for analysis.

4.2.2 Stimuli and design

Compared to Experiment 1 (Chapter 3), Experiment 2 shows a number of differences. The major ones are highlighted through headings in italics throughout this section and rationale for each is provided. *(i) Area covered by*

the stimulation. Instead of applying the stimuli from the inner to the outer arm, I applied them from the shoulder to the elbow (proximo-distally). With this change I wanted to resemble as much as possible episodes of naturalistic stroking. Indeed, it emerged both from informal conversations with caregivers and from observing episodes of stroking in our PCIs, that this is the preferred and most common way of stroking limbs. The hand condition was similar to Experiment 1: on average 5 - 6 strokes every 10 seconds. In the toothbrush condition the Experimenter stroked the baby's arm at a speed > 10cm/s (35 strokes every 10 seconds on average) (Figure 4.1a). *(ii) Baseline length*. In addition, in contrast to Experiment 1, the baseline lasted 20 seconds[19] rather than 10 seconds. I extended the length of the baseline because I observed in Experiment 1 that the HbO_2 response sometimes had not returned to baseline levels by the end of the following trial. I believe this was because stimulation probably extended beyond the end of the trial if a stroke began towards the end of the 10 seconds interval and was not interrupted; because the following baseline trial would still follow the timing set via the audio cues it therefore become shorter in length. Therefore, I extended the length of the baseline trials in Experiment 2 to 20s to ensure that the baseline trial exceeded 10s and I had therefore allowed the haemodynamic response to return to baseline levels by the end of each trial. (iii) Side of stimulation. Another important difference with Experiment 1 is the side of stimulation. I tested the first 20 subjects applying both stimuli to the same arm and counterbalancing the side of the stimulation (like in Experiment 1). However, to my surprise preliminary results showed a large response to the non-affective stimulus and a lack of response to the affective one. This could have been because the vibrating toothbrush could have saturated the response of receptors over the area of application and therefore lessened the response to the following hand stimulation. Thus, for the remaining 22 infants, I decided to deliver the two conditions on different arms.

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[19] 12 of the 21 infants were presented with longer Experimental trials lasting for 20s each. This step, alongside change of application direction, was initially taken to mimic naturalistic episodes of gentle stroking. Data analyses focused on the first 10s of the trials to mirror the period of stimulus presentation used in Experiment 1.

As in Experiment 1, stimulus presentation timing was controlled by audio signals via headphones. (iv) order of presentation of the stimuli. Given that the hypothesized difference in activation (hand > spoon) was not evident in Experiment 1 I took steps to ensure that this finding was not caused by the design, such as a predictable pattern of stimulus presentation. A fixed ABBA BAAB ABAB BAAB sequence was therefore used to prevent participants from expecting any regularity. Overall the total number of trials per condition was the same as in Experiment 1, and in line with the procedure of Experiment 1 I counterbalanced the first trial (hand/toothbrush) and order of presentation across participants. The electric toothbrush was kept on for the duration of the study to ensure the sound remained continuous and did not cue the infants alongside the touch stimulus or lead to increased cross-modal activation.

4.2.3 Procedure

I followed the same procedure as described in Experiment 1[20]. Measurements from this group of infants showed that the average head circumference was 42.85 cm (SD = 1.31). For a summary of total presented and total valid trials see Table 4.1.

Figure 4.1 a) Experimental design Experiment 2: the stroking was performed using a toothbrush or a hand; Experimental trials were 10 seconds long and baseline trials were 20 seconds long. b) Experimental design Experiments 3&4: the stroking was performed using a soft brush either at a slow (5cm/s) or a fast (30cm/s) speed; Experimental trials were 10 seconds long and baseline trials were either 10 or 15 seconds long. c) A schematic showing the location of the channels relative to the 10-20 coordinates (in red) for Experiment 2. d) A schematic showing the location of the channels relative to the 10-20 coordinates (in red) for Experiments 3&4. The location of channels 1-26 is the same in both Experiments.

4.2.4 Data processing and analysis

I followed similar analysis steps to those used in Experiment 1 to reject channels, convert intensity to concentration data and reject invalid Experimental trials. In contrast to Experiment 1, the block used to remove linear trends in the data during the de-trending process differed in length due to the differences in trial lengths across the Experiments. It follows that the data was segmented into blocks of 34 seconds of data, consisting of 4 seconds of the baseline prior to the onset of the tactile stimulation, 10 seconds of tactile stimulation, plus the following 20 seconds' baseline. Each block of attenuation data was de-trended with a linear fit between the average of the first (-4 to 0s) and the average of the last 4 seconds (26 to 30s) to remove drifts in the signal. In addition, for those infants (12 out of 21) with 20s Experimental trials and 20s baseline trials we de-trended between -4 to 0 and 36 to 40s.

For this Experiment, we used the same analysis plan as for Experiment 1. Those channels that showed a response to either touch and survived FDR corrections were paired with the homologous channel in the opposite hemisphere and analysed using LMMs. Hemisphere (right, left), stimulus (hand, toothbrush) and time-window (1-5s, 5-9s) were entered as repeated measures factors, and side of stimulation (right, left) as between-subjects factor.

	Experiment 2	Experiment 3	Experiment 4
n	21	20	16
age (days)	158.84(15.96)	230.52(24.4)	332.61(19.77)
female/male	9:12	9:10	10:6
head circumference (cm)	42.85(1.31)	44.36(1.61)	45.19(2.34)
number of trials completed	12.90(3.16)	18.94(6.73)	16.5(4.44)
valid trials	11.42(3.10)	16.26(5.7)	15.18(4.3)
valid trials in affective touch condition	5.85(1.82)	7.68(2.7)	6.66((3.16)
valid trials in non-affective touch condition	5.57(1.66)	8.57(3.06)	7.16(2.59)
number of rejected channels per infant	3.9(2.9)	6.68(9.09)	9.25(10.27)

Table 4.1 Participants' information for Experiment 2 and Experiment 3 and 4. The number of valid trials refers to the number of trials included in the analysis after off-line coding of the infant's behavior during the study. The first number refers to the mean value across the group and the bracketed number refers to the standard deviation.

4.3 Results Experiment 2

In an initial channel-by-channel analysis of the fNIRS data, t-tests compared the grand averaged hemodynamic peak changes in HbO_2 and HHb (during the same time windows of activation as in Experiment 1) evoked by the hand and the toothbrush compared to baseline. Here I report only the channels that showed significant increases in HbO_2; significant decreases in HHb did not survive FDR correction for either condition. In this study, the hand condition revealed no significant activation in any of the channels, in either time-window (see Figure 4.2 top panel). In contrast, the toothbrush condition revealed left lateralized activation in five channels. Three channels (ch. 4, 5, 26) showed a significant response in the early time-window and two (ch. 9, 11) had significant activation in both time windows (see Figure 4.2 bottom panel). All channels reported here survived FDR correction, while a complete list of corrected and uncorrected significant responses can be found in Table 4.2. In this age-group these channels are positioned approximately over IFG (Ch.4), the inferior frontal-precentral gyrus (Ch. 5) and pSTS-TPJ region (Ch. 9, 11, 26) in the left hemisphere (Lloyd-Fox et al., 2014). Time-courses in Figure 4.3 clearly show an absence of a response to the hand, even in channels where the hand elicited a significant increase in HbO_2 in Experiment 1 (Ch. 3 and 9). These time courses also show that, despite a left lateralization of the activation to the toothbrush, the two channels in the right hemisphere also show a sustained response (Figure 4.3, Ch. 14 and 20), even though these do not meet significance thresholds.
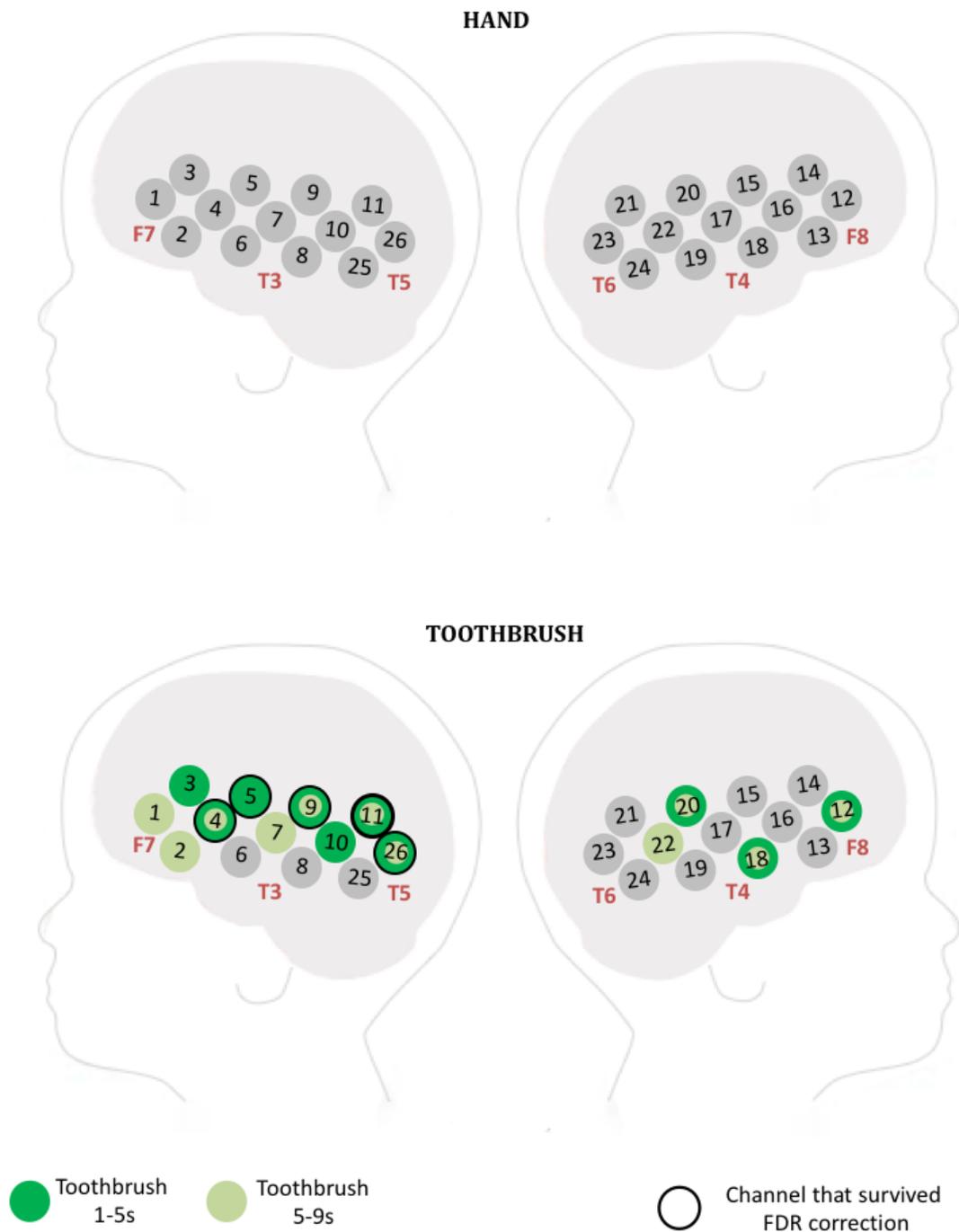
Figure 4.2 A schematic view of the NIRS arrays showing HbO₂ responses to the hand (top panel) and to the toothbrush (bottom panel). Channels marked in dark green revealed a significant response in the 1-5s time-window to the toothbrush versus baseline; channels marked in pale green revealed a significant response in the 5-9s time-window. Channels marked in both colours had a significant response over both time windows. A black circle is placed around those channels that survived FDR corrections

To investigate hemispheric differences between the two conditions, each of the channels showing significant HbO_2 response to either stimuli was paired with the homologous channel in the opposite hemisphere, resulting in five pairs: pair1 (ch. 16 and 4), pair2 (ch. 20 and 9), pair3 (ch. 15 and 5), pair4 (ch. 21 and 11), pair 5 (ch. 26 and 23). Each pair was analysed using LMMs.

For Pair 1 (IFG), a main effect of stimulus was found (F(1,26.267)=13.491, p=.001) with greater activation to the toothbrush (M=.658, SE=.181) compared to the hand (M=-.129, SE =.158). I also found a significant interaction between hemisphere and stimulus (F(1,22.592)=6.392), p=.019. Post-hoc t-tests revealed no significant hemispheric differences for either the response to the hand (t(18)=1.687, p=.112) or for the response to the toothbrush (t(18)=1.276, p=.219). When contrasting the response to the two stimuli within the same channel I found a significantly larger response to the toothbrush compared to the hand in the left (t(18)=3.454, p=.003) but no difference in the right hemisphere (t(18)=1.245, p=.230).

For pair 2 (pSTS-TPJ), I found a main effect of stimulus (F(1,26.988)=23.856, p=<.001), with greater activation to the toothbrush (M=.881, SE=.190) compared to the hand (M=-.287, SE =.188), and a significant interaction between hemisphere and stimulus F(1,21.046)=7.827, p=.011). Post-hoc t-tests revealed that there are no hemispheric differences neither for the response to the hand (t=.410, p=.688) nor for the response to the toothbrush (t=.089, p=.930). When contrasting the response to the two stimuli within the same channel I found a significantly larger response to the toothbrush compared to the hand in both Ch.9 (t=3.426, p=.004) and Ch. 20 (t=3.515, p=.003). I also found a significant interaction between hemisphere and side of stimulation (F(1,23.520)=76.923) p<.001) and a three way interaction between hemisphere, side of stimulation and stimulus (F(1,26.656)=5.802 p=.023). The challenge in following up the hemisphere*side of stimulation interaction is that I cannot collapse across conditions since some of the infants (n=12) during the same session received the stimuli on separate arms. For these infants, I cannot average the response to the hand and to the toothbrush in the same hemisphere. I therefore followed this interaction in the group of infants that received the stimuli on the same arm (n=9). In this group paired samples t-tests showed that for those infants that received the stimuli on

the right arm, activation in the left hemisphere was larger compared to the right hemisphere (t(3)=4.61, p=.044, d=2.66), whereas stimulation on the left arm led to a larger response in the right hemisphere (t(4)=3.978 p=.016, d=1.77). The three-way hemisphere*side of stimulation*stimulus interaction was first followed up in the group that received the stimuli on the same arm. Post-hoc t-tests revealed that for those infants that received the stimuli on the right arm the response was contralateral to the toothbrush (t(3)=5.384, p=.016) whereas no hemispheric difference was found to stimulation with the hand. For those infants that received the stimuli on the right arm, while there was no difference between hemispheres for the hand, the hemispheric difference for the toothbrush did not reach significance (p=.08). Findings from this subgroup of infants suggest that the contralatrality of these responses is driven by the toothbrush stimulus.

For Pair 3 (precentral gyrus), I found a marginal main effect of hemisphere F(1,21.797)=4.277, p=051, with greater HbO_2 increases in the left (M=.218, SE=.181) compared to the right hemisphere (M=-.338, SE=.232) and a main effect of stimulus F(1,35.423)=4.486, p=.041 with greater activation to the toothbrush (M=.207, SE=.178) compared to the hand (M=-.326, SE =.224). Furthermore, the interaction between hemisphere and side of stimulation was significant (F(1,29.968)=5.921, p=.021). This interaction was followed up using the same approach used for pair 2. However, post hoc t-tests revealed that in neither group hemispheric differences reached significance (ps>.12).

For pair 4 (pSTS-TPJ), I found a main effect of stimulus (F(1,24.728)=28.717 p<.001, with greater activation to the toothbrush (M=1.049, SE=.168) compared to the hand (M=-.068, SE =.175). There was also a significant interaction between hemisphere and side of stimulation (F(1,31.659)=10.815 p=.002). Post-hoc t-tests were run following the approach used for pair 2. For the group of infants that received the stimulation on the right arm the response to the toothbrush was contralateral to the side of stimulation (larger response in left vs right hemisphere) (t(3)=2.27, p=.05, d=1.13) but for those infants who received the stimulation on the left arm there were no hemispheric differences (p=.35). The interaction between side of stimulation and stimulus was also significant (F(1,24.524)=10.361 p=.004),

suggesting that a larger activation was observed in the contralateral hemisphere in response to the toothbrush.

For pair 5 (pSTS-TPJ), I found a main effect of hemisphere $F_{(1,20.032)}=6.199$ p=.022 with greater HbO_2 increases in the left (M=.668, SE=.153) compared to the right hemisphere (M=.138, SE=.248). I also found a significant interaction between hemisphere and side of stimulation $F_{(1,30.653)}=16.995$ p<.001. Post-hoc t-tests were run following the approach used for pair 2. A contralateral response was observed for those infants that received the stimulation on the right arm (t(3)=3.73, p=.016, d=1.88) but it did not reach significance for those who received the stimulation on the left arm (p=.33). A three way interaction between hemisphere, side of stimulation and stimulus was also significant ($F_{(1,26.944)}=14.939$ p=.001). As for pair 2, this interaction was unpacked in the group that received the stimuli on the same arm. A significant contralateral response to the toothbrush was found both for infants who received the stimulation on the right arm (t(3)=2.33, p=.05, d=1.16) and for those who received it on the left arm (t(2)=4.198, p=.026, d=2.42). The response to the hand did not differ across hemisphere in either group (ps>.31).

Despite the fact that I unpacked the two- and three- way interactions running post-hoc analyses only on a subgroup of infants (those that received the stimuli on the same arm) the consistency of these results across pairs of channels suggests that, in this experiment the toothbrush elicits contralateral responses in channels located over the pSTS/TPJ (Ch 20-9, 21-11, 26-23).
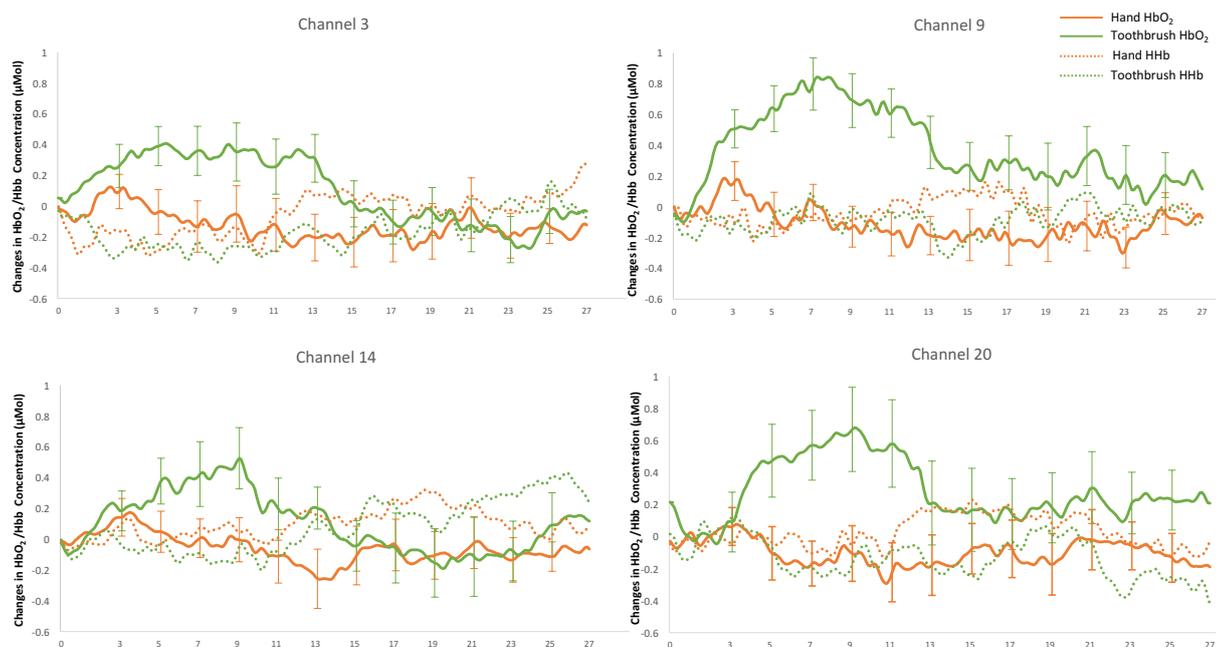
Figure 4.3 Grand averages of haemodynamic time courses within channels that showed significant responses, and are centered within two key areas known to respond to social touch: IFG (Ch. 3 left and Ch. 14 right) and pSTS-TPJ (Ch. 9 left and Ch. 20 right) Error bars represent standard error

4.4 Discussion Experiment 2

In light of the similar responses elicited by the hand and spoon stimuli found in Experiment 1 possibly due to the stimuli being too similar to one another, I asked if moving the physical properties of the two stimuli farther apart would have led to a clearer differential response in the cortical areas under investigation (pSTS/TPJ and IFG).

In Experiment 2 where the non-affective stimulus differed from the affective stimulus across four physical dimensions (texture, speed, temperature and vibration), the results were unexpected. Firstly, I found that, when compared to the baseline, the toothbrush produced similar robust responses as the hand and spoon had in Experiment 1. These encompassed the IFG, the inferior frontal-precentral gyrus and pSTS-TPJ region in both hemispheres. While FDR corrected channel by channel analyses suggest a left-lateralized response, the LMM analysis only revealed a main effect of hemisphere in one of the pairs of channels suggesting that the toothbrush elicits an equally strong response in both hemispheres. This is further confirmed when following the interactions between stimulus and hemisphere over both IFG and pSTS.

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Furthermore, responses to the toothbrush were shown to be contralateral to side of stimulation.

Secondly, I found no significant differences from baseline for the hand stroking. These surprising findings cannot be ascribed to peripheral habituation or to the fatiguing of the CT afferents in the limb, as the same pattern of results was observed both in the infants that received the stimulation on the same arm and in those that received hand and toothbrush on different arms. However, it is possible that the lack of hand activation was a consequence of the toothbrush stimulation itself. Evidence shows that stimulation with high frequency vibration can cause an attenuation in central excitability, depressing the detection thresholds for other tactile stimuli presented in temporal proximity (Macefield & Burke, 1991). Therefore, it is possible that the non-affective stimulus inhibited or altered responses to the hand that, in Experiment 1, had elicited clear responses. I have to note however that, in a recent study on adults, skin stroking elicited stronger activations than skin vibration (applied with a static stimulus continuously for 15 seconds) in several brain regions, including the left IFG, but responses to the two stimuli were alike in pSTS (Davidovic et al., 2016). Interestingly, stroking, compared to vibration, elicited larger responses even in the somatosensory cortices, whilst a larger response to vibration was not found in any region. These findings could suggest that the vibration of the toothbrush on its own might not be responsible for the altered response to the affective touch, but rather it's the combination of high speed, rough texture and vibratory motion that could explain my finding. Alternatively, vibration may have a more powerful effect in infancy, diminishing even responses to highly relevant social stimulation.

Another finding that differentiates Experiment 2 from Experiment 1 is the contralaterality of the responses. Indeed, these results indicate that responses to the toothbrush are contralateral to the side of stimulation, while this was not true for either stimuli in Experiment 1. It is interesting that a contralateral response is elicited by the most intense stimulation thus far explored. This seemingly incidental finding might help to shed light on the true nature of this unexpected response. Is it plausible that the responses I observe do not result from the direct recruitment of IFG and pSTS/TPJ for the processing of the social properties of the touch, but that instead these originate

elsewhere and what I am observing is the extension of a nearby non-social response? I suggest that the toothbrush elicits strong activation in the primary somatosensory cortex (over the postcentral gyrus close to the midline where the upper arm is represented[21]) and that, due to the intensity of the stimulus, this extends onto the nearby cortices that I happen to be recording from. Two pieces of evidence support this argument. The first is that a contralateral response to tactile stimuli is observed in the somatosensory cortex from 2 months of age using EEG and MEG (Rigato et al., 2014, 2017; Saby et al.,2015; Meltzoff et al., 2018a; 2018b)[22]. The second is that vibration was reported to elicit widespread responses in 2-to-9 days old infants (Shibata et al., 2012). Indeed, using fNIRS it was shown that stimulation with a buzzer (continuously for 10 seconds) on the hand leads to strong widespread responses over temporal and centro-parietal areas (Shibata et al., 2012). While in this study recordings were limited to the somatosensory cortex, the authors suggest that given the intensity of the observed response this may have extended onto adjacent areas. The tactile experience of a newborn is of course very different from that of a 5-month-old, but prolonged vibration could be processed in a similar fashion across early development. It is possible that it takes extensive experience before the brain shows the diminished response to vibration observed in adults (Davidovic et al., 2016) and future research should investigate the development of these responses. While some EEG studies on somatosensory processing also used vibrotactile stimuli (e.g. Rigato et al 2015, 2017), I cannot compare these findings with mine due to the differences in duration of stimulation (in the order of hundreds of milliseconds in Rigato et al.) and to the less localized response measured with EEG.

Another possibility for a strong response to the toothbrush over the pSTS/TPJ region is that this is caused by multimodal matching. It is possible that matching the sound of the electric toothbrush to its touch leads to the recruitment of this region, involved in multimodal processing. Also, I cannot

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[21] A somatotopic representation of the body in the somatosensory cortex has been reported from 2 months of age (Meltzoff et al., 2018; Saby et al., 2015).

[22] Contralateral responses to tactile stimuli possibly emerge during the first two months of postnatal life since in newborns evidence seems to converge towards a lack of lateralization (Nevalainen et al., 2008; Erberich et al., 2006; Bartocci et al., 2006).

exclude that infants know that it is a human administering the touch as they are aware that the experimenter is there. Thus, this activation could reflect an incongruency response to the type of touch and the administrator.

Overall Experiment 2 did not support my hypothesis. While I thought that the approach of differentiating as much as possible the affective from the non-affective stimulus would have facilitated a discriminatory response in favour of the affective stimulus over social brain regions, it actually led to a response in the opposite direction to the expected one. A plausible explanation for such findings lies in my choice of the non-affective stimulus. Nonetheless a complete explanation is still missing as I cannot tease apart the individual contribution that speed, texture and vibration had on the observed responses.

In experiment 3 and 4 the affective and the non-affective stimuli differ only on one physical dimension: speed. If the faster speed of the toothbrush did not cause the unexpected pattern of findings observed in experiment 2, the new contrast should be better situated to reveal differential responses to affective and non-affective touch in infants.

	Experiment 2											
	Hand> Baseline							**Toothbrush> Baseline**				
Ch	*HbO$_2$/ HHb*	*TW*	*t*	*p*	*df*	*d*	**Ch**	*HbO$_2$/H Hb*	*TW*	*t*	*p*	*df*	*d*
4	HHb	1-5	-2.96	0.009	17	-0.64	1	HbO$_2$	5-9	2.23	0.038	18	0.49
							2	HbO$_2$	5-9	2.73	0.014	17	0.6
							3	HbO$_2$	1-5	2.66	0.017	17	0.58
							4*	HbO$_2$	1-5	3.17	0.005	18	0.69
							4	HbO$_2$	5-9	2.45	0.025	18	0.54
							5*	HbO$_2$	1-5	3.09	0.006	19	0.67
							7	HbO$_2$	5-9	2.44	0.027	15	0.53
							9*	HbO$_2$	1-5	3.9	0.001	16	0.85
							9*	HbO$_2$	5-9	3.6	0.002	16	0.78
							10	HbO$_2$	1-5	2.34	0.034	14	0.51
							11*	HbO$_2$	1-5	6.95	<0.001	16	1.52
							11*	HbO$_2$	5-9	4.61	<0.001	16	1.01
							12	HbO$_2$	1-5	2.28	0.035	18	0.5
							12	HbO$_2$	5-9	2.83	0.011	18	0.62
							18	HbO$_2$	1-5	2.43	0.033	11	0.53
							18	HbO$_2$	5-9	2.75	0.019	11	0.6
							20	HbO$_2$	1-5	2.32	0.033	17	0.51
							20	HbO$_2$	5-9	2.74	0.014	17	0.6
							22	HbO$_2$	5-9	2.48	0.025	15	0.54
							26*	HbO$_2$	1-5	5.12	<0.001	16	1.12
							26	HbO$_2$	5-9	2.58	0.02	16	0.56
							24	HHb	1-5	-3.65	0.003	12	-0.8
							24	HHb	5-9	-3.66	0.003	12	-0.8
							26	HHb	5-9	-2.24	0.039	16	-0.49

Table 4.2. Significant activations from baseline in Hand and Toothbrush conditions (Experiment 2). * indicates that the response survived the false discovery rate (FDR) correction

4.5 Methods - Experiment 3 and 4

4.5.1 Participants

Two age-groups were tested in this study. Twenty 7-month-old infants took part in Experiment 3 (9 females; mean=230.52 days, SD=24.4, range=186-272 days). A further thirteen infants participated but were excluded from the study owing to fussiness (n = 2), or a high level of rejected data due to motion artifact (n = 11).

Sixteen 10 month old infants took part in Experiment 4 (10 females; mean=332.61 days, SD=19.77, range=281-370). A further fourteen infants participated but were excluded from the study owing to fussiness (n = 5), or a high level of rejected data due to motion artifact (n = 9).

This study has been carried out at Keio University (Tokyo, Japan). Parents gave informed consent in compliance with a protocol approved by the ethic committee of Keio University, faculty of letters (14034-0-2).

4.5.2 Stimuli and design

Each stimulus trial was 10 seconds long (Figure 4.1b). The affective touch condition consisted of a gentle stroke, performed at 3cm/s, performed by the experimenter on the baby's forearm with a soft brush, with repeated stroking applied proximo-distally for 6 cm in length between the elbow to the wrist. In the non-affective touch condition, brush stroking was delivered at approximately 30cm/s. All participants received the stimulation on the right arm. To time the presentation of the stimuli, the Experimenter listened to audio cues played in headphones which indicated not only the onset and offset of each trial but also the length of the individual strokes (a tone played every 2 s for the affective touch condition and every 0.2 s for the non-affective touch condition). The audio cues were provided by SuperLab5 (Cedrus Corporation), which also placed event markers into the fNIRS recordings online. The affective touch trial consisted on average of 5 strokes while the non-affective touch trial consisted on average of 50 strokes. Following each 10 second trial there was a period of no- touch baseline which randomly lasted either 10 or 15 seconds. The conditions (affective/non-affective) were presented in a pseudorandom order.

During the procedure participants watched an animated video with sound on an iPad or played with toys to avoid them orienting to the tactile stimulation.

4.5.3 Apparatus

Infants wore Hitachi headgear consisting of two source-detector arrays containing a total of 44 channels (source-detector separations: 20 mm). The arrays were placed over both hemispheres. In addition to the areas covered by the CBCD arrays described in Chapter 3 (inferior frontal - temporal lobes) these probes further covered the middle frontal gyri and a portion of the parietal lobes (see Figure 4.1d). The headgear used was the same for 7 and 10 month olds. Data was collected with the NIRS system ETG-7000 (Hitachi, Tokyo, Japan). This system used 2 continuous wavelengths of source light at 780 and 830 nm. Before the infants began the study, measurements of their head circumference, ear to ear lateral semi circumference, and nasion to inion were taken, and the location of the channels and arrays relative to these anatomical landmarks were recorded (Sarah Lloyd-Fox et al., 2014). Measurements from the younger group of infants showed that the average head circumference was 44.18 cm (SD = 1.71), while for the older group it was 45.41 cm (SD=2.25).

4.5.4 Procedure

The infants were held on their parent's lap in front of a table, on the other side of which the second experimenter was sitting. The parent was asked to refrain from interacting during the stimuli presentation unless the infant became fussy or sought their attention. The main experimenter sat next to the infant and delivered the stimuli to the infant's arm being careful to remain out of the infant's sight. In order to avoid that infants turned to the right and looked at the experimenter and the stimuli, the second experimenter showed the infants an animated video with sound (consisting of Japanese nursery rhymes) on an iPad, and she engaged them with toys in cases where they lost interest in the video. The experiment ended when the infants became fussy. Each session was recorded using a video camera placed to the left side of the participant, and infant behaviour was coded offline.

4.5.5 Data processing and analysis

4.5.5.1 Pre-processing using Homer2

In contrast to Experiments 1 and 2, for the preprocessing of these datasets I used Homer2 (Huppert, Diamond, Franceschini, & Boas, 2009), an open source software for fNIRS data pre-processing. The first reason behind this choice lies in the differences between the raw data file formats obtained with the NTS and with the ETG-7000 NIRS systems. The in-house programs used for preprocessing in Chapter 3 were developed to work with the NTS data format and would have needed considerable adjustments in order to be used with a different file format. Conversely Homer2 can accommodate any NIRS data file following initial conversion to a readable format. Secondly, this choice mirrors a trend in the field, as Homer2 is being used by an increasing number of research groups (e.g., Lloyd-Fox et al., 2015; Ravicz et al.,2015; Miguel et al.,2017; Timeo et al., 2017) who are moving away from their in-house fNIRS data processing software options.

4.5.5.2 Motion Correction

A main difference compared to Experiments 1 and 2 is that, instead of discarding trials affected by motion artifacts, I applied motion correction techniques to recover as many trials as possible. The approach I took to motion correction in this chapter was informed by extensive work done to test the performance of different motion correction techniques on infants' as well as semi-simulated data. This work (Di Lorenzo et al., 2018 submitted for publication) was motivated by a lack of guidelines on how to approach motion correction with infant data, since all motion correction techniques had been validated on adult data. I am joint first author in this work and I have worked on two of the infants' datasets (there were three in total): one dataset involved an audiovisual paradigm and the other a touch paradigm (data from Experiment 4 of this Chapter were used for the analysis).

Motion artifacts in infant data are far more complex compared to those recorded in typical adults. In adult experiments movements are usually kept to a minimum by simply asking participants to avoid head movements. Therefore, artifacts are relatively rare and also relatively easy to identify in a dataset. On

the contrary, infants cannot be instructed to remain still and, apart from limited cases (i.e. sleeping infant), motion artifacts typically affect the entire recording. Movements occur for example during bouts of fussiness, boredom or even excitement; the variability and the unpredictability of these behaviors, coupled with their high frequency of occurrence during the recording, makes it harder to identify their effects and correct the resulting artifacts that corrupt infant data.

Therefore in Di Lorenzo et al., we compared the performance of spline interpolation (Scholkmann et al., 2010) wavelet filtering (Molavi & Dumont, 2012) (both recommended by work on adults' data- Brigadoi et al., 2014; Cooper et al., 2012) both individually and in combination. This choice was driven by the variability of motion artifacts typically seen in data from young participants: combining two techniques that target different types of motion artifacts should outperform the use of each technique on its own. Performance of these different motion correction methods was tested on four different datasets (collected at different research labs, with different tasks, age-groups, headgears and NIRS acquisition systems). One of the datasets used for this analysis is data from the older infants in this chapter. Results from this work showed that the combination of the two techniques recovered most of the trials affected by motion artifacts, reduced the two quality metrics tested (between and within subjects standard deviation), and better recovered the true HRF in semi-simulated data. In light of these findings I adopted the same approach to motion correction for data analysis in this chapter.

The reason for applying motion correction to only two of the three datasets presented in this chapter is that data from Experiment 2 were preprocessed using in-house programs that do not include this option. At the same time, I could not preprocess these data using Homer2 with the pipeline used for Experiment 3 and 4. This is because in Experiment 2, 12 of the 21 infants were presented with longer Experimental trials lasting for 20s each (vs. 10s). The in-house programs gave me the flexibility to consider only the first 10 seconds of stimulation for these infants but the same was not possible in Homer2. While I strongly advocate the use of motion correction when dealing with infants fNIRS data, I believe that applying motion correction in Experiment 2 would not lead to significant changes in the reported results. The benefits

would be that each infant could contribute to the final analysis a few more trials and that some of the infants (n=6) that were initially excluded because of a high level of rejected data due to motion artifacts could be included in the analysis, but it is unlikely that this would change the observed pattern of responses.

4.5.5.3 Data Processing

fNIRS data were processed using Homer2 software package. *Behaviour-based trial rejection.* I first manually excluded trials from the analysis according to the same criteria used for Experiment 1 and 2. A minimum of three trials per condition was necessary to include the participant in the analysis.

Channel rejection. The first function of the processing stream was enPruneChannel, which automatically identifies and excludes from further analysis those channels showing very high or low optical intensity readings. Channels that survive this step and have a good intensity, can still be affected by large amounts of noise and sometimes need to be excluded. While it is possible to exclude these based on whether they exceed a predefined value of signal-to-noise ratio, I could not satisfactorily fine-tune this parameter and channels that mostly had a good signal ended up being automatically discarded because of noise confined to a small portion of the recording. Therefore, I manually excluded data or channels according to the following rules: If a channel reached saturation values over a short period of time (i.e. max 2 trials), the data corresponding to the noise is rejected using 'exclude time'[23]; while if the channel reached saturation values for a more prolonged period of time (> 2 trials), the channel was excluded. Some channels were also excluded because of technical issues during the recording that affected their signal. After this step, the raw intensity data were converted to optical density changes.

Motion correction. I first applied hmrMotionArtifact, a function that identifies and flags artifacts based on predefined values[24]. This was necessary

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[23] This procedure involves manually marking the time points where data is deemed to be invalid. Any stimulus markers contained within the marked time points will not be included in the HRF calculation.

[24] hmrMotionArtifact is a function that detects the signal exceeding a threshold in change of amplitude (AMPthresh) or/and a threshold in change of standard deviation (SDEVthresh) within a predefined time-window (tMotion) and marks as artifacts the data points around the detected motion (+/- tMask). All parameters are defined by the user.

for the correct functioning of Spline interpolation, which acts, channel by channel, on previously detected motion artifacts. The spline function corrects the artifact by performing a cubic spline interpolation of the artifact; the interpolation is then subtracted from the original signal. After this, the signal is baseline corrected to ensure that signal time-course before and after the corrected artifact is continuous. In Homer2, spline interpolation depends on a parameter (p) that can be set by the user; in this study, we used p = 0.99, the same value used by previous studies (Scholkmann et al., 2010; Cooper et al., 2012; Brigadoi et al., 2014). To the signal already corrected with the spline interpolation I applied wavelet filtering. This function decomposes the signal time-course of every channel in a series of wavelet detail coefficients which are characterized by a Gaussian distribution: while the coefficients linked to the physiological components (NIRS signal of interest) will be distributed around zero, the coefficients reflecting motion artifacts can be identified as the outliers of the Gaussian distribution. Then, by setting to zero all detail coefficients identified as outliers of the distribution (< first quartile - α times the interquartile range or > third quartile + α times the interquartile range) and reconstructing the signal with the modified coefficients (with the inverse discrete wavelet transform), we can obtain a version of the original signal with a much-reduced presence of motion artifacts. In Homer2 the α threshold can be defined by setting the tuning parameter iqr. For this study, we used iqr = 0.8, which was defined by visually inspecting the effects of this and other iqr values on the group-averaged HRFs (i.e., 1.2, 1.0, 0.5).

Motion- artifacts-based trial rejection. Following the correction of motion in the signal with first spline and then wavelet, hmrMotionArtifact was used to identify what artifacts were still present. Using enStimRejection, trials are automatically discarded if an artifact falls during the 2 seconds preceding the stimulation or during the stimulation itself.[25] *Filtering.* After this step, a band-pass filter (third order Butterworth) with a passband of 0.025–1 Hz was applied

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[25] In this step, the trials corrupted by motion artifacts are rejected from all the channels and not on a channel-by-channel basis as in the pre-processing Experiments 1 and 2. Following this step infants are removed from the group analysis if they are not left with at least three trials per condition.

to reduce slow drifts[26] and high-frequency noise. Following this the optical density data were converted to concentration changes using the modified Beer–Lambert law (Cope & Delpy, 1988; Delpy et al., 1988) with a differential pathlength factor of 5.1 (Duncan et al., 1995). Finally, all remaining trials were block-averaged for every condition, channel and participant. The length of each block was 20 seconds long, including 10 seconds of stimulus and 10 seconds of baseline. Chromophore concentrations were baseline corrected using the 2 s prior to stimulus presentation, as in previous fNIRS studies (for example Ravicz et al., 2015). While in experiment 2 detrending was performed using the average of the 4s prior to stimulus presentation, here I only used the last 2 seconds. Since in Experiment 3 the baseline was shorter than in Experiment 2 (10/15s vs. 20s) using 2 instead of 4s to baseline correct was a cautious step to make sure that in this segment the HRF had returned to baseline level.

EnPruneCh	dRange	9e-01 4e+00
	SNRthresh	0
	SDrange	0.0 45.0
	reset	0
hmrMotionArtifactbyChannel	tMotion	1.0
	tMask	1.0
	STDEVthresh	13.5
	AMPthresh	0.4
hmrMotionCorrectSpline	p	0.99
hmrMotionCorrectWavelet	iqr	0.8
hmrMotionArtifact	tMotion	1.0
	tMask	1.0
	STDEVthresh	13.5
	AMPthresh	0.4
enstimRejection	tRange	-2.0 10.0
hmrBandPass	hpf	0.025
	lpf	1
hmrOD2Con	ppf	5.1 5.1
hmrBlockAvg		-2.0 20.0

Table 4.3 Homer2 processing options used for the analysis of Experiment 3 and 4.

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[26] The high pass filter serves the same function (removing slow drifts in the signal) as the detrending procedure applied to the data in Experiments 1 and 2.

4.5.5.4 Statistical Analysis

Preliminary analysis. Statistical analysis has been performed outside of Homer2, since the software does not yet have this functionality. A preliminary channel-by-channel analysis was run using in-house MATLAb programs (developed by a collaborator - Dr. Katherine Perdue, Boston Children's Hospital, Harvard Medical School) to identify those channels that responded to touch, irrespective of condition. This was achieved by comparing the response to the Experimental trials (for specific time-windows; see below) with the response during the pre-stimulus signal (2 seconds pre-onset) across all infants, using the valid data for each channel. The time windows selected matched those used in Experiment 1 and 2 (1-5s and 5-9s). In addition we also ran exploratory analyses in two later time windows (9-13s and 13-17s) to investigate the latency of the response, given the difference in age tested across Experiments. Statistical comparisons (two tailed t-tests) were performed, to compare the maximum signal change during the specified Experimental trial time windows, with the averaged pre-stimulus signal.

Preliminary analyses showed that no channel survived multiple comparisons in either Experiment. This suggests that, despite correcting for motion artifacts, the signal-to-noise ratio was much lower in these two datasets compared to those collected at the CBCD. This is likely due to a combination of factors. In this study, one experimenter performed the tactile stimulation on the infants' arm and another one distracted them with a video and toys. It is therefore likely that the ecological nature of this setting induced the infant to move more than in the other two experiments. There was also a relatively high number of optodes (30 vs. 20), and the infants were more likely to have dark, thick, hair characteristic of this population during infancy. Increased optodes and hair would likely lead to greater instability of the headgear on the head. More movement coupled with a less tightly fitting headgear resulted in a larger number of motion artifacts in these datasets. Even though still managed to correct for motion artifacts and recover a number of trials sufficient for the analyses, the recovered segments of data had a lower signal-to-noise ratio that those from the previous datasets.

For Experiment 3, in order to investigate effects of hemisphere, stimulus and time window I used the results from the previous experiments as a guide. I started by selecting those channels that in Experiment 1 and 2 had shown a response to any type of touch and that survived FDR correction[27]. I obtained a total of 7 pairs of channels. I then ran a LMM on these pairs of channels together with results from Experiment 2. The reason for analysing experiment 3 together with experiment 2 (and not with experiment 1) was because their experimental designs were similar due to the shared speed contrast. In both experiments, slow stroking was contrasted to fast stroking. In order to analyse how the response unfolds over time with different stimuli past the stimulus offset (10s), two further time-windows were extracted for Experiment 2 (9-13s, 13-17s). Hemisphere (right, left), stimulus (affective, non-affective) and time-window (1-5s, 5-9s, 9-13s, 13-17s) were entered as repeated measures factors, and Experiment (2, 3) as between-subjects factor.

For Experiment 4 I could not adopt the same approach (combining this with the dataset from Experiment 2) due to the age difference across groups (5 and 10-month-old). Indeed, across samples, the same pair of channels would not represent responses originating from similar brain regions. In their work where fMRI and fNIRS data from 55 infants were coregistered, Lloyd-Fox and colleagues found that age was a factor in the location of underlying anatomy from 4 – 7 months. Therefore, I took a cautious approach not to directly compare 5 and 10 month olds as the location of pairs of channels would likely differ across these groups. Despite the age difference across Experiment 4 and 3 was smaller (7 and 10-month-old), data from these two experiments were not directly compared with LMMs as, again, I could not be certain that underlying brain regions for each pair of channels would be similar across groups. Another reason for not directly comparing these groups was the different sample size, as for each pair of channels fewer infants would have had data for Experiment 4 compared to Experiment 3.

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[27] This was possible since channels 1 to 26 In Experiment 3 should overlay the same portion of cortex as channels 1-26 in Experiment 1 and 2 (given that the age-groups are similar, and the source-detector separation and procedure for alignment of the headgear to external scalp landmarks was identical across studies).

Therefore, for this dataset I just ran the channel-by-channel analysis. I report those channels where activation to any condition versus baseline was significant at the .05 alpha level. In order to locate the cortical regions that correspond to these channels I cannot refer to the map of channel locators outlined by Lloyd-Fox and colleagues (2014) since this can be used with participants between 4 and 7 months of age. Previous infants' work (Imafuku et al., 2014; Xu et al., 2017) that used the same 3x5 Hitachi probe holder used in the current study defined channels locations based on the virtual registration method devised by Tsuzuki et al. (2007). This method consists in the placement of a virtual probe holder on the scalp by simulating the holder's deformation and by registering probes and channels onto the canonical brain template in the standard stereotaxic coordinate system. Although this estimation was originally devised for the adult brain, Imafuku and colleagues (2014) argue that this can also be applied to infant's data. This is possible because the source detector separation in the infants' studies is of 20 mm (instead of 30 mm employed by the spatial registration for adults). As infants' head circumference is approximately one third of that of adults, having a source-detector separation one third shorter than the one used in adults results in similar relationship between the probe positions and brain areas of infants and adults, because the relative scalp position between infants and adults is not different (Barkovich et al., 1988; Minagawa-Kawai et al., 2008). Furthermore, examining infant's MRI anatomical images of the brain, Matsui et al. (2014) reported that macroanatomical structures were generally comparable between adult and infant atlases, indicating that the virtual registration employed for fNIRS can be also reliably applied to infant brain.

4.6 Results Experiment 3

In an initial channel-by-channel analysis of the fNIRS data, t-tests compared the grand averaged hemodynamic peak changes in HbO_2 and HHb (during four time windows: 1-5s, 5-9s, 9-13s, 13-17s) evoked by slow and fast touch compared to baseline. No channels survived FDR correction for either condition. Given the exploratory nature of this study, we also report those channels that showed significant activation at the .05 alpha level, prior to FDR correction. For the slow

versus baseline contrast, one channel (Ch.22) showed a significant HbO^2 increase in two of the four time-windows investigated (5-9 and 9-13s- see Figure 4.4 top panel) and three channels (Ch. 21, 22, 39) showed significant HHb decrease. In this age-group these channels are positioned approximately over pSTS-TPJ region (Ch. 21, 22) (Lloyd-Fox et al., 2014). It is possible that Channel 39 was located over the somatosensory cortex, but this is an approximate estimation and we cannot have the same degree of confidence that we have for the location of channels 1-26 (the position of channels 1-26 on the cortex has been defined for this age-group in NIRS-MRI co-registration work by Lloyd-Fox et al., 2014). In contrast, the fast touch condition revealed HbO$_2$ increase in eleven channels. Five of these (ch. 17, 21, 31, 39, 42) showed a significant response in at least one of the first two time-windows, while the remaining six (Ch. 9, 11, 20, 22, 32) had significant activation in the later time windows (see Figure 4.4 bottom panel). Two channels (Ch. 25, 31) had significant HHb decreases. In this age-group these channels are positioned approximately over the inferior frontal-precentral gyrus (Ch. 17) and pSTS-TPJ region (Ch. 9, 11, 20, 21, 22, 25) (Lloyd-Fox et al., 2014). Given the vicinity of channel 42 with channels 20, 21, 22, this is likely over the same pSTS/TPJ region. As described above, it is possible that Channel 39 (and 31) could be located over the somatosensory cortex. A complete list of significant responses can be found in Table 4.4.
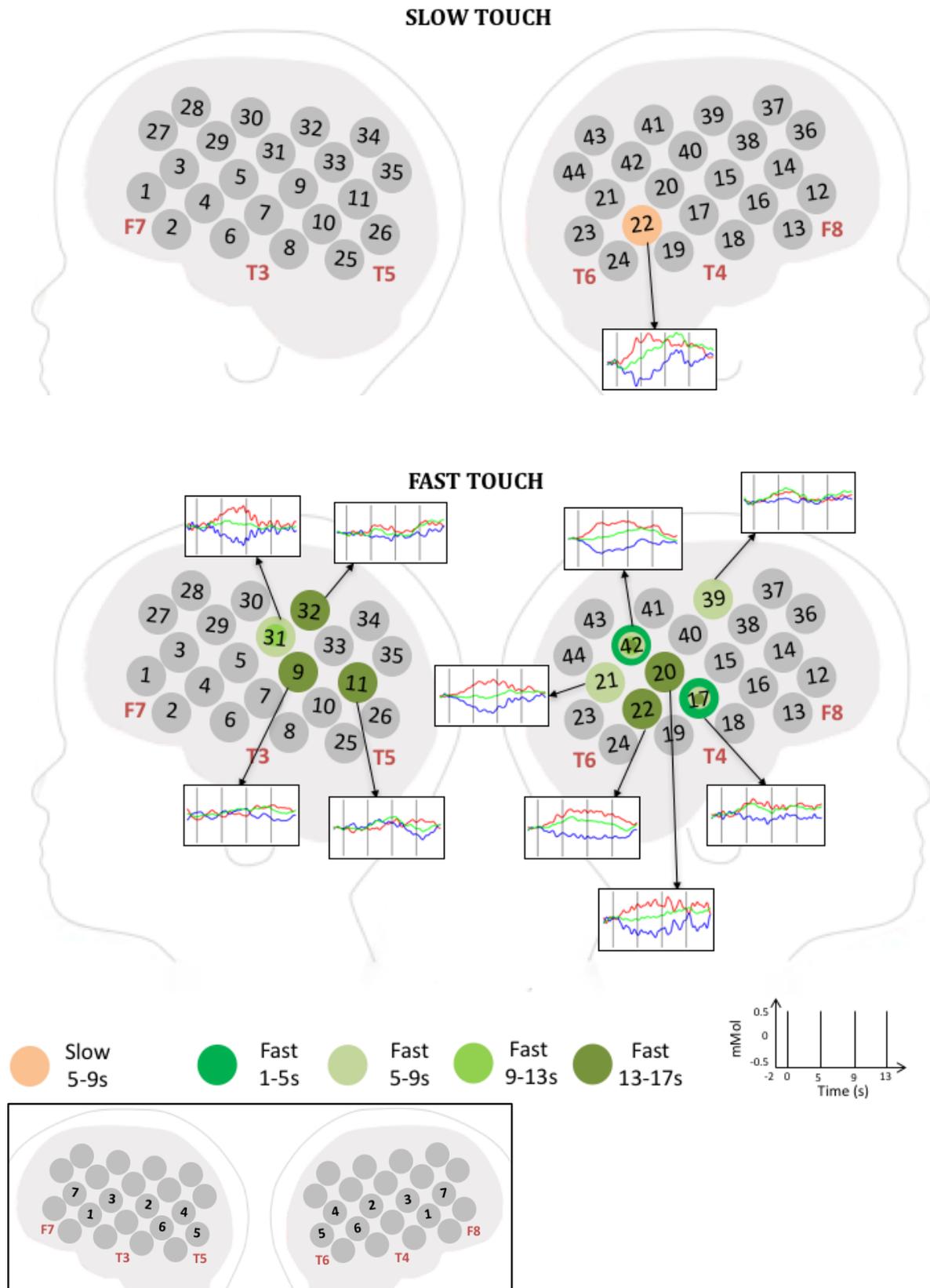
Figure 4.4 A schematic view of the NIRS arrays showing HbO₂ responses to slow (top panel) and to fast touch (central panel). Significant responses versus baseline are reported for the four time windows investigated. Next to each significant channel, its time course is reported. HbO₂ is represented in red, HHb in blue and total haemoglobin in green. At the bottom of the figure, a panel showing those channels that form the seven pairs for the LMMs

	Experiment 3												
	Slow> Baseline							**Fast> Baseline**					
Ch	*HbO2/HHb*	*TW*	*t*	*p*	*df*	*d*	**Ch**	*HbO2/HHb*	*TW*	*t*	*p*	*df*	*d*
22	HbO2	5-9	2.52	0.024	15	0.63	9	HbO2	13-17	2.97	0.009	15	0.74
22	HbO2	9-13	2.59	0.020	15	0.65	11	HbO2	13-17	3.04	0.008	16	0.74
22	HHb	1-5	-2.70	0.016	15	-0.68	17	HbO2	1-5	2.38	0.031	15	0.60
21	HHb	1-5	-2.18	0.045	15	-0.55	17	HbO2	5-9	2.85	0.012	15	0.71
21	HHb	9-13	-2.36	0.032	15	-0.59	17	HbO2	13-17	3.85	0.002	15	0.96
39	HHb	1-5	-3.52	0.003	15	-0.88	20	HbO2	13-17	2.66	0.019	14	0.69
							21	HbO2	5-9	2.17	0.046	15	0.54
							22	HbO2	13-17	3.25	0.005	15	0.81
							31	HbO2	5-9	2.72	0.015	17	0.64
							31	HbO2	9-13	2.62	0.018	17	0.62
							32	HbO2	13-17	2.33	0.033	16	0.57
							39	HbO2	5-9	2.22	0.042	15	0.56
							42	HbO2	1-5	2.65	0.018	15	0.66
							42	HbO2	5-9	2.34	0.034	15	0.58
							42	HbO2	9-13	2.17	0.046	15	0.54
							42	HbO2	13-17	3.41	0.004	15	0.85
							25	HHb	1-5	-0.50	0.048	15	-0.13
							31	HHb	9-13	-2.15	0.046	17	-0.51

Table 4.4 Significant activations from baseline in Slow and Fast touch conditions

in Experiment 3

To investigate the effects of stimulus, hemisphere and time-window across Experiments 2 and 3, a LMM was run for 7 pairs of channels. Any interaction with hemisphere not specific to experiment 3 could not be explored. For example, the three-way interaction *hemisphere*stimulus*experiment* would be followed within each experiment and if significant differences were found within experiment 2 but not 3 these would not be reported. This is because in Experiment 2 hemisphere was associated with side of stimulation (contralateral responses) and this interaction could not be exhaustively unpacked due to the heterogeneity of side of stimulation.

For pair 1 (16-4; IFG), I found a main effect of stimulus $F_{(1,36.905)}=5.535$, p=.024, with greater activation to the non-affective stimulus (M=.195, SE=.091) compared to the affective stimulus (M=-.094, SE =.083).

For pair 2, (20-9; pSTS-TPJ), I found a main effect of stimulus $F_{(1,33.669)}=37.276$, p<.001, with greater activation to the non-affective stimulus (M=.473, SE=.107) compared to the affective stimulus (M=-.095, SE =.079). I also found a significant interaction between experiment and stimulus $(F_{(1, 33.669)}=22.176, p<.001)$. Post-hoc t-tests revealed that in Experiment 2, the non-affective stimulus elicits more activation than the affective one $(t_{(19)}=5.038, p<.001)$ whereas in Experiment 3 the difference between stimuli was not significant $(t_{(17)}=1.124, p=.277)$. Comparing the same stimulus across studies revealed opposite responses. Indeed, this pair of channels responded more to affective touch in Experiment 3 than in Experiment 2 $(t_{(31.49)}=2.877, p=.006)$ whereas the opposite holds true for non-affective touch with larger responses in Experiment 2 $(t_{(25.524)}=2.822, p=.009)$. The three way interaction experiment*time-window*stimulus was also significant $(F_{(1,28.637)}=5.211, p=.005)$; this is be discussed below.

For pair 3 (15-5; precentral gyrus) I found no main effects or significant interactions (all ps>.089).

For pair 4 (21-11; pSTS-TPJ) I found a main effect of stimulus $(F_{(1, 28.977)}=24.943, p<.001)$, with greater activation to the non-affective stimulus (M=.398, SE=.089) compared to the affective stimulus (M=-.019, SE =.105). A significant interaction between experiment and stimulus $(F_{(1,35.056)}=11.574, p=.002)$ was followed up with post-hoc t-tests. As for pair2, in Experiment 2, the non-affective stimulus elicited more activation than the affective one $(t_{(18)}=3.751, p=.001)$ whereas in Experiment 3 the difference between stimuli was not significant $(t_{(19)}=1.213, p=.240)$. A comparison across Experiments showed that responses to non-affective touch are larger in Experiment 2 vs. 3 $(t_{(27.252)}=2.234, p=.034)$, whereas responses to affective touch are larger in experiment 3 vs.2 $(t_{(26.687)}=1.994, p=.05)$. The three way interaction experiment*time-window*stimulus was also significant $(F_{(1, 35.242)}=4.836, p=.005)$; this is be discussed below.

For pair 5 (26-23; pSTS-TPJ) I found no main effects or significant interactions (all ps>.065).

For pair 6 (22-10; pSTS-TPJ) I found a main effect of stimulus (F(1,26.621)=7.615, p=.010), with greater activation to the non-affective (M=.285, SE=.110) compared to the affective stimulus (M=-.105 SE =.073).

For pair 7 (14-3; IFG), no main effects and no significant interactions were found (all ps>.099).

4.6.1 Time effects over pSTS/TPJ

For two pairs of channels over pSTS/TPJ I found a significant three-way interaction between experiment, time-window and stimulus. To unpack this interaction, I first compared responses in each time-window to the same condition across experiments (e.g. hand stroking Experiment 2 vs. slow brush stroking Experiment 3). Next I compared responses within each experiment across affective and non-affective stimuli.

For Pair 2 (ch.9-20), across experiments affective touch elicits a significantly larger response in experiment 3 vs. 2 between 5-9s (t(36)=2.709, p=.010) and between 9-13s post stimulus onset (t(31.795)=2.070, p=.042) (Figure 4.5, top left panel). Differences to the non-affective stimuli are due to the fact that while in experiment 3 the response increases gradually over the 4 time windows, in experiment 2 an abrupt onset (1-5s) and an early peak (5-9s) are followed by a steady decrease to baseline levels (9-17s). Significant differences were found between 1-5s (t(27.350)=2.74, p=.011) and between 5-9s (t(27.350)=2.63, p=.014) (Figure 4.5, bottom left panel).

Within Experiment 2 activation to non-affective touch is significantly larger than to affective touch between 1-13s, with no differences between 13-17s, as here the response has returned to baseline levels (1-5s: t(19)=4.35, p<.001; 5-9s: t (19)=6.65, p<.001; 9-13s: t (19)=3.62, p=.002). In contrast, within Experiment 3 affective and non-affective touch show a similar time-course between 1-13s and they differ between 13-17s, where the response to affective touch returns to baseline while that to non-affective touch continues to increase (t(17)=3.11, p=.006) (Figure 4.5, right panel).

Figure 4.5 Time courses of HbO$_2$ changes to affective and non-affective touch stimuli across Experiment 2 and 3 for four time windows: 1-5, 5-9, 9-13, 13-17 s post stimulus onset. Responses have been averaged across channels 9 and 20 (pSTS/TPJ) in order to unpack the experiment*stimulus*time-window interaction. **Top left panel**: responses to affective touch in experiment 2 (red) and 3 (orange). **Bottom left panel**: responses to non-affective touch in experiment 2 (dark green) and 3 (bright green). **Right panel**: responses to affective (orange) and non-affective touch (green) in experiment 3. Significant differences within a time-window (the .05 uncorrected alpha level) are marked with an asterisk. Error bars represent standard error

For pair 4 (ch.11-21) responses show a similar pattern as for pair 2. Indeed, across experiments affective touch elicits a significantly larger response in experiment 3 vs. 2 between 9-13s post stimulus onset (t(31.081)=2.331, p=.026)(Figure 4.6, top left panel). Similarly to pair 2, non-affective touch elicits significantly larger responses in experiment 2 vs. 3 between 1-5s (t(30.216)=2.96, p=.006) and between 5-9s (t(29.469)=2.69, p=.012) (Figure 4.6, bottom left panel).

Within Experiment 2 activation to non-affective touch is significantly larger than to affective touch between 5-13s (5-9s:t (18)=3.93, p=.001; 9-13s:t (18)=3.77, p=.001). Within experiment 3 affective and non-affective touch show

a similar time-course across all four time windows (Figure 4.6, right panel). While a trend similar to pair 2 emerges (response to affective touch returning to baseline while response to non-affective touch continuing to increase between 13-17s), this does not reach significance.

Figure 4.6 Time courses of HbO$_2$ changes to affective and non-affective touch stimuli across experiment 2 and 3 for four time windows: 1-5, 5-9, 9-13, 13-17 s post stimulus onset. Responses have been averaged across channels 11 and 21 (pSTS/TPJ) in order to unpack the experiment*stimulus*time-window interaction. **Top left panel**: responses to affective touch in experiment 2 (red) and 3 (orange). **Bottom left panel**: responses to non-affective touch in experiment 2 (dark green) and 3 (bright green). **Right panel**: responses to affective (orange) and non-affective touch (green) in experiment 3. Significant differences within a time-window (the .05 uncorrected alpha level) are marked with an asterisk. Error bars represent standard error.

4.6.2 Summary results experiment 2 and 3

In Experiment 2 channel-by channel analysis revealed only responses to the non-affective stimulus versus baseline and no activation to affective touch (vs. baseline). This was confirmed by linear mixed models ran for five pairs of channels (over IFG, pSTS and the precentral gyrus). In each pair, non-affective touch elicited a larger response than affective touch. Surprisingly affective touch elicited no response at all in this experiment.

In Experiment 3 channel-by channel analysis revealed a similar pattern of responses as the one observed in Experiment 2. Indeed, while only one channel showed activation to affective touch (versus baseline), ten channels showed activation to non-affective touch. Linear mixed models were experiment 2 and 3 were analysed together revealed that the non-affective>affective was a pattern common to both experiments. However post-hoc analysis performed for four channels positioned over pSTS-TPJ revealed that in Experiment 3 the affective and the non-affective stimulus elicited similar responses which only differed in a late time window (13-7s post stimulus onset).

The comparison across experiments also showed that responses to different stimuli had significantly different time courses. While activation to the toothbrush (Experiment 2) is fast, limited to the presentation of the stimulus and returns to baseline levels as soon as stimulation ends, activation to brush stroking (Experiment 3) increases slowly and is protracted post stimulus offset. Notably activation to fast touch remains sustained even after the response to slow touch has returned to baseline.

4.6 Results Experiment 4

In an initial channel-by-channel analysis of the fNIRS data, t-tests compared the grand averaged hemodynamic peak changes in HbO_2 and HHb (during four time windows: 1-5s, 5-9s, 9-13s, 13-17s) evoked by slow and fast touch compared to baseline. No channels survived FDR correction for either condition. To follow are those channels that showed significant activation at the .05 alpha level, prior to FDR correction. Slow touch (versus baseline) elicited a significant HbO_2 increase in two channels (Ch. 19 and 35- see Figure 4.7) and significant HHb decreases in three channels (Ch. 7, 9, 23). In contrast, fast touch elicited no significant HbO_2 increases in any channels, and significant HHb decreases in 3 channels (Ch. 2, 9, 10). A complete list of responses can be found in Table 4.5.

The virtual registration method (Tsuzuki et al., 2007) was used for spatial estimation of underlying brain regions. This method was previously adopted when the same Hitachi array was used. According to this method, responses to slow touch are located over the middle temporal gyrus (Ch. 7, 23), the angular gyrus (Ch.35, 9) and the precentral gyrus (Ch.19). HHb responses to fast touch are located over the frontal inferior operculum (Ch.2), the middle temporal gyrus (Ch.10) and the angular gyrus.
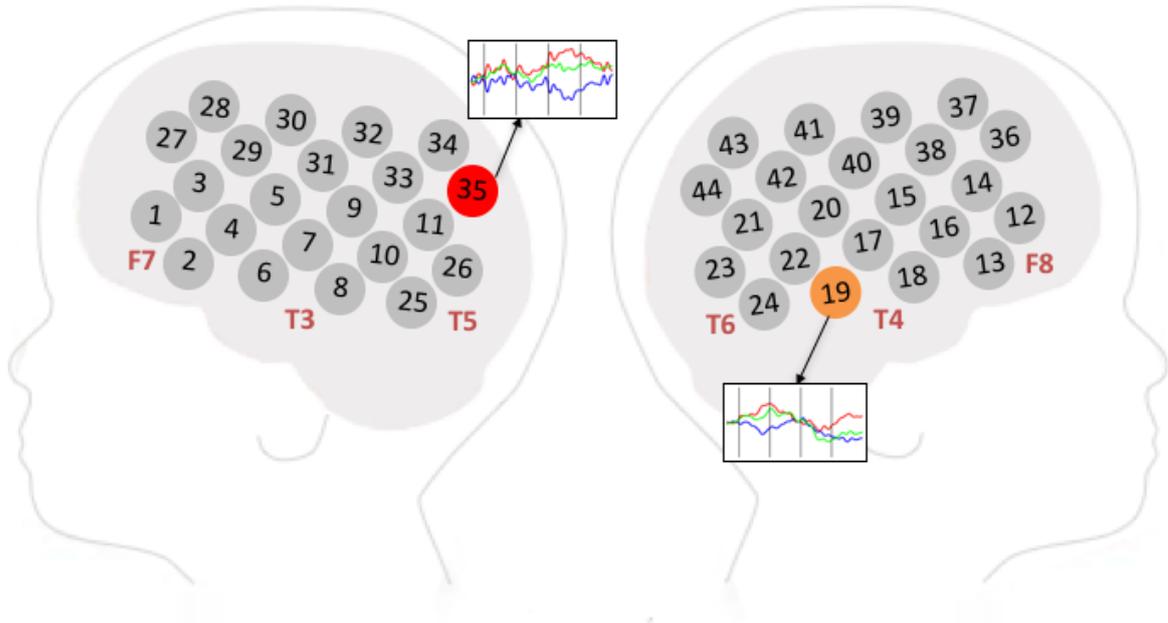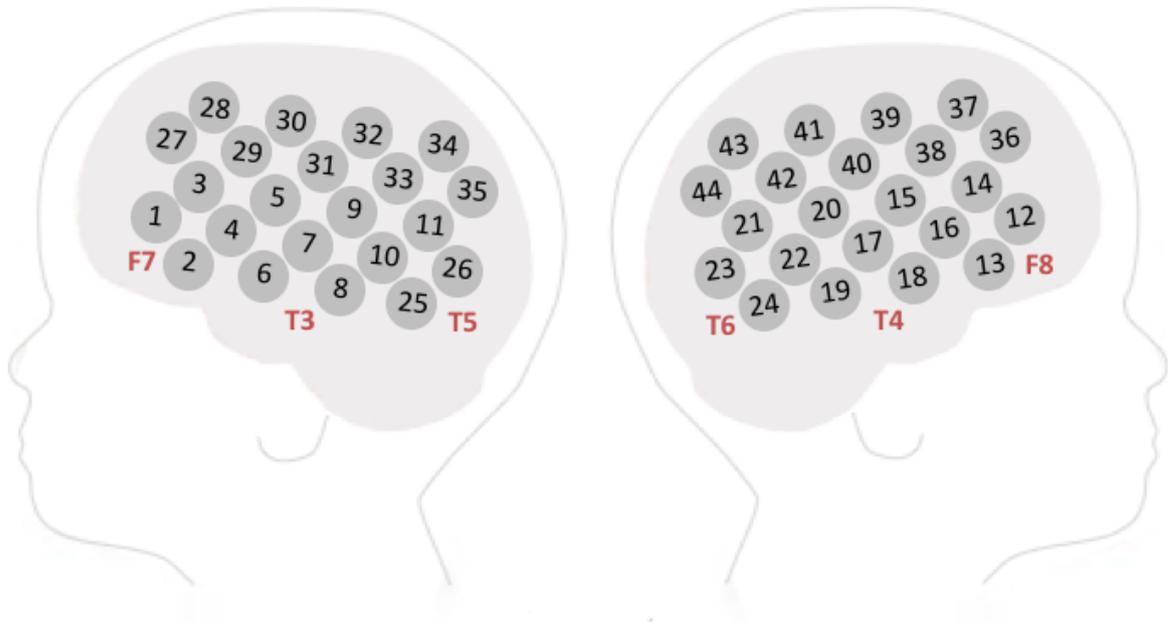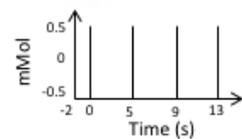
Figure 4.7 A schematic view of the NIRS arrays showing HbO$_2$ responses to slow (top panel) and to fast touch (bottom panel). Significant responses versus baseline are reported for the four time windows investigated. Next to each significant channel, it's time course is reported. HbO$_2$ is represented in red, HHb in blue and total haemoglobin in green

			Experiment 4										
		Slow> Baseline							Fast> Baseline				
Ch	HbO_2/HHb	TW	t	p	df	d	**Ch**	HbO_2/HHb	TW	t	p	df	d
19	HbO2	1-5	2.43	0.038	9	0.77	2	HHb	9-13	-2.44	0.035	10	-0.74
35	HbO2	13-17	3.09	0.011	10	0.93	9	HHb	9-13	-2.49	0.028	12	-0.69
7	HHb	1-5	-2.84	0.016	11	-0.82	10	HHb	1-5	-2.87	0.015	11	-0.83
7	HHb	9-13	-2.24	0.046	11	-0.65							
9	HHb	9-13	-2.18	0.050	12	-0.61							
23	HHb	1-5	-2.49	0.038	8	-0.83							

Table 4.5 Significant activations from baseline in Slow and Fast touch conditions

in Experiment 4.

4.7 Discussion

The experiments in the present chapter had the aim to expand and clarify the findings from Chapter 3. In light of the similar responses elicited by the hand and spoon stimuli found in Experiment 1 possibly due to the stimuli being too similar to one another, I asked if moving the physical properties of the two stimuli farther apart would have led to a clearer differential response in the cortical areas under investigation (pSTS/TPJ and IFG). I explored two contrasts, one where the stimuli differed along four dimensions (Experiment 2), and the classical one of slow vs. fast brush stroking adopted in adult's studies of affective touch (Experiments 3 and 4). The advantage of having data from two different contrasts is that it informs us on how responses vary across different sets of stimuli. Specifically, are the responses to the controlled brush stimulation replicated when stimuli with different textures are used? If stroking with a brush and with the human hand are both perceived as affective touch, similar responses are expected. Otherwise, different responses in the social cortical areas under investigation would indicate that the two stimuli are not processed as similar, and the use of a brush in infants' studies should be questioned.

Experiment 2 revealed an unexpected pattern of findings, with large and broadly distributed responses to the non-affective stimulus (the electric toothbrush) and no significant responses in any channel to the affective

stimulus (hand-stroking). In the discussion following experiment 2 I concluded that a plausible explanation for these findings lies in my choice of the non-affective stimulus, although I cannot reach a satisfactory explanation as the individual contribution that speed, texture and vibration had on the observed responses cannot be teased apart.

In contrast to Experiment 2, in Experiment 3 a classical slow vs. fast touch contrast was used. One advantage of this contrast compared to the previous one is that the affective and the non-affective stimuli only differ along one physical dimension: speed. Further, slow (as compared to fast) brush stroking has been successfully employed in adults' studies to show CT afferents activation (Ackerley et al., 2014; Loken et al., 2009), and discriminatory neural and physiological responses (Voos et al., 2013; Morrison et al., 2011). The same contrast has been used successfully in infancy, with slow touch eliciting activation over the insula and the posterior temporal cortex in 2-month-old infants (Jönsson et al., 2017) and heart rate decreases in 9-month old infants (Fairhurst et al., 2014). Despite the change of stimulation, Experiment 3 yielded once again an unexpected pattern of responses. While the response to slow touch (versus baseline) was confined to one channel over the pSTS/TPJ region, fast touch (versus baseline) elicited a stronger and more widespread response over the same area. Whilst this interesting pattern emerged from the preliminary channel-by-channel analysis, no channel survived FDR corrections suggesting that these responses are weaker than those reported from the previous experiments. Furthermore, while combining data from Experiment 2 and 3 revealed an overall larger response to the non-affective stimulus, this was driven by the toothbrush>hand difference and a direct comparison between slow and fast touch failed to reveal any significant differences. Interestingly from LMMs it emerged that in two channels over pSTS/TPJ the response to slow and fast touch unfolds in a similar way, but while for slow touch (between 13-17s post stimulus onset) it returns to baseline levels, for fast touch it continues to increase. Out of the 7 pairs of channels investigated with LMMs, only one revealed this difference between slow and fast touch. It is interesting that response to the non-affective stimulus elicits a response that protracts long after the end of the stimulation. Could fast touch be perceived as a more novel stimulation and thus be processed for longer? Still it is unclear why both

affective and non-affective touch are processed in a similar way in a region dedicated to social processing. Another finding from the post-hoc analyses over these channels revealed that the responses to the non-affective stimuli across experiments had very different time courses. Indeed, while response to fast touch develops gradually and continues to increase after the end of the stimulation, response to the toothbrush has a rapid increase, an early peak and returns to baseline levels as soon as the stimulation ends. Since fast speed is common to both stimuli, this difference could be due to the features of toothbrush stroking that are not in common with fast brush stroking, i.e. vibration and rough texture.

Despite the fact that from the channel-by-channel analysis a widespread response to fast brush stroking was observed, no difference with slow touch emerged in any of the seven pairs of channels analysed with LLMs, besides the difference described above, found in one pair for a late time-window. A possible reason is that signal-to-noise ratio was much lower in Experiment 3 compared to the Experiment 2 and I identify three factors that could have contributed to this difference. First, the setting of Experiment 3 was more naturalistic than the one employed in Experiment 2, and the presence of a person facing the infant could have elicited movements aimed to interact with her. Second, it is likely that the Hitachi headgear used in Experiment 3 was less tightly fitting on the head than the custom-built CBCD headgear used in Experiment 2. The Hitachi headgear had a higher number of optodes (30 versus 20) and a different design was used to hold the probes (net of cloth strings versus silicone band). Third, hair colour and thickness differed across the samples tested in the UK and in Japan, with more variance of hair type in the UK compared with a prevalence of dark hair in the latter sample, which would certainly play a role in explaining the lower quality of the recorded signal. Therefore, a higher occurrence of movements, combined with a less tightly fitting headgear and the presence of dark hair could account for the differences in signal-to-noise ratio between the two experiments. To the best of my knowledge no previous study used with infants the same headgear used here in Experiments 3 and 4. In two studies that measured activation over the prefrontal cortex in infants using a 3x5 array (as the ones used for this study), responses were strong and some channels survived FDR corrections (Imafuku et al., 2014; Xu et al., 2017). However, in

both instances, although the same array was employed, it used one and not two arrays (thus halving the total number of optodes on the infants' head) which would certainly have led to a tighter optode-scalp fit and to higher signal-to-noise-ratio.

Notwithstanding the weaker responses observed using brush stroking the pattern that emerges from the channel-by-channel preliminary analysis is certainly one worthy of consideration. We can, at first glance, appreciate a resemblance with findings from Experiment 2. Indeed, across stimuli contrasts, the non-affective stimulus elicits a widespread activation compared to the affective one, which either elicits no activation (in the case of hand stroking) or activation restricted to one channel only (in the case of slow velocity brush stroking). This result is in itself rather surprising. Changing stimulus contrast did not bring about a response in line with the hypothesis, it instead revealed a near replication of the previous findings. I had thought that stimulation with the toothbrush, due to its complex physical properties, had caused the paradoxical response to hand stroking. However, the fact that a similar pattern is observed when only one dimension (speed) is manipulated evokes a somewhat different explanation. Results reported thus far would indicate that certain physical properties of tactile stimulation interfere with the processing of affective touch diminishing the cortical response to it. These results point at fast speed, common to both toothbrush and soft brush stroking, as a source of such interference while suggesting that cold temperature does not afford the same effect.

Despite similarities, there are clear differences between responses to the toothbrush and those to fast brush stroking. The fact that in Experiment 3 activation is not contralateral to side of stimulation, suggests that it does not originate in the somatosensory cortex, as I propose is the case for the response to the toothbrush. Instead, fast brush stroking elicits an activation that is localized over the area that I had hypothesized to be selective to affective touch, the pSTS/TPJ. The fact that this area clearly responds to non-affective touch persuades me to think that its selectivity to the affective properties of touch has not developed yet.

In Experiment 3, the additional optodes (not common to those used in Experiment 2) located over the parietal cortex allowed me to investigate

somatosensory responses. While fast touch seems to elicit these responses bilaterally, the same does not hold true for slow touch.   This is surprising given that Miguel et al., (2017) found that both slow stroking and static touch activated the contralateral somatosensory cortex in a similar age group using fNIRS. These results suggest that fast stroking interferes with slow stroking dampening the response even in the primary sensory cortex.

One question that arises is: why could I not replicate the same findings as Jönsson and colleagues (activation in STS to slow touch) despite similarities with Experiment 3 in stimulus contrast? One difference between their experiment and mine is that they had a shorter stimulus presentation (2s) and longer inter stimulus interval than I did (length was random with mean of 31s). Possibly had I allowed a longer interval between non-affective touch I would have not observed this interference. Future work should manipulate baseline length to confirm this. A further possibility is that in Jönsson et al. infants were in a swaddled hug in their parents' arms facing their parent. Therefore, the stroking could have been perceived as if the parent was stroking them.

Drawing together the threads from these first three experiments, I put forward some concluding thoughts. While the results of Experiment 1 indicate that cortical specialization to affective touch is already emerging by 5 month of age (following the interpretation that infants perceived both stimulations as affective touch), recording responses to non-affective touch in a similar age-group over this same area in Experiment 3 is instead suggestive of the fact that this specialization is still underway. Furthermore, results from Experiments 2 and 3 suggest that between 5 and 7 months of age the perception of affective touch is subject to interference from tactile stimulation with certain properties. Fast velocity seems to be the most common factor between stroking with the brush and with the toothbrush, whereas the effects specific to texture and vibration should be isolated in future studies. Interestingly for this interference to take place the stimulations do not need to be concurrent (because I still observe it when affective touch is applied 10-20 seconds after the end of non-affective touch) nor to be delivered to the same location on the skin (separate arms were stimulated in Experiment 2). Whilst not in line with my hypothesis, these findings open the way to future research investigating the interaction between tactile stimuli of different intensities.

In this age-group affective touch elicits inferior frontal and posterior temporal activations, but this response seems to be neither specific to affective touch (as I observe it also with control stimulation) nor stable. During the period of time that it takes these regions to fine-tune their responses to the affective qualities of touch, some paradoxical responses are observed. The question remains whether this phenomenon is due to a lack of specialization in these cortical regions or whether, from an evolutionary standpoint, it makes sense that in the presence of vigorous tactile stimulations gentle touch is no longer processed (at the cortical level). For example, if an intense tactile stimulation signaled danger, the entire organism would be tuned to attend to it and to mobilize resources to face it. Somatosensory stimulation signaling danger could have originated either from the environment or from a conspecific. To put forward one example, if the mother perceived a situation of danger she would grab her pup tight to her and run to find a safe place, thus generating intense tactile stimulation. Esposito and colleagues showed that when rodent pups are carried by their mothers they show a calming response measured with a heart rate decrease, decrease of ultrasound vocalizations and immobilization (Esposito et al., 2013). Is it possible that the maternal tight grasp and somatosensory stimulation elicited by fast motion signals threat and blocks sensory processing of stimuli not relevant in that moment? If the tight grasp is what triggers the pup to hold the position that will ensure success of the maternal rescue (and eventually their own safety) it might be important that the pup continues processing this stimulation over any other competing, not immediately relevant, stimulations.

By testing 10-month-old infants, in Experiment 4 I aimed to shed light on the developmental trajectory of these responses. Caution is needed when interpreting these results as these i) come from a smaller sample size, and ii) none of the channels in the channel-by-channel analysis survived FDR corrections (the same reasons outlined for the lower SNR in Experiment 3, apply to this dataset as well). Despite these caveats, results are nonetheless noteworthy. Firstly, slow touch stimulation elicits activation in the same region (neighboring channels) as in Experiment 3. Secondly, and most importantly, the response to fast touch is no longer present in this portion of cortex (besides the decreases in HHb). While the response to slow touch is consistent across the

two age-groups tested, the response to fast touch greatly diminishes with age. An unexpected finding is that somatosensory responses are not observed to either touch. One would have expected responses in pSTS/TPJ and IFG to tune to affective touch, with no changes in the somatosensory responses.

Taken together these findings seem to indicate that near the end of the first year of life, the response to affective touch over pSTS/TPJ stabilizes, while responses to non-affective touch in the same region subside. Similarly to processing of social stimuli in the auditory modality, these findings suggest that specialization to tactile social stimuli takes longer to develop, as compared to social visual stimuli. Perhaps months of substantial relevant experience of social and non-social stimuli in these modalities are necessary for the social brain to tune its responses to social stimulation. However not having observed specialization in the cortical regions investigated here (IFG and pSTS) does not rule out the possibility that other nodes of the social brain (e.g. the medial prefrontal cortex) displayed selective responses to affective touch in these experiments.

In Chapter 1 I had asked whether the role social touch plays in socio-emotional development is mediated by activation of the social brain. If selectivity for social touch in the social brain emerges towards the end of the first year of life, then probably this is not what mediates the positive effects observed in younger infants. However, these effects were usually observed in an interactive situation, with the parent in front of the baby. Therefore, it is possible that, at least during the first months of life, this network of regions is engaged only in a rich context with other types of social cues. This could explain the fact that a response was observed in Jönsson et al. and not in Miguel et al. or in the experiments presented in this and the previous chapter.

In the study by Jönsson and colleagues, the infants were held in their parents arms and they could see their parent and the stroking action on their arm. This contrasts with the experimental setup of the current studies, where I intentionally removed other cues so as to investigate the unique contribtion that temperature and speed have, in the processing of affective touch. In previous fNIRS research investigating temporal lobe activation to communicative cues in a similar live setting, higher activation was found when a combination of visual and auditory ostensive singals were used (Sarah Lloyd-

Fox et al., 2015). One possibility is that specialization to individual components of social stimuli develops slowly and is facilitated by exposure to multi-modal input. Auditory or tactile stimuli might need to be experienced in conjunction with their visual manifestation (i.e. someone talking to or caressing the child), for enhanced responses to be evident in pSTS, a region described as a multi-modal hub. It may also be that, at least as specialization develops in childhood, the presence of multi-modal information is necessary for selective responses to be observed in experimental situations. Selective responses have been observed in adults to isolated presentation of affective touch (e.g. Gordon et al., 2013; Voos et al., 2013). However, it is prescient that this response is highly sensitive to top-down cognitive factors (e.g. who is providing the touch; for a review see Ellingsen et al., 2016).

4.7.1 Limitations

An inherent limitation to using fNIRS is the fact that I could only measure responses to touch from the surface of the cortex. Therefore I don't know whether the posterior insula, a region involved in processing affective touch in children, adolescents and adults, (e.g. Bjornsdotter et al., 2014; Olausson et al., 2010) is selective to affective touch in 5-months-old infants; as it is folded deep within the lateral sulcus and cannot be reached by near infrared light emitted at the scalp level. In infancy, insular activation in response to slow stroking was recently reported both in newborns using fMRI (Tuulari et al., 2017) and in 2-month-old infants using diffuse optical tomography (DOT) (Jönsson et al., 2018). Therefore, it's possible that a discriminatory response was present at the depth of the insula, but that the technique used for the present study did not allow us to measure it.

A further limitation of fNIRS, is represented by the number of channels one chooses to record from. While it is desirable to simultaneously record responses from different cortical regions using the highest possible number of channels, this comes at the cost of negatively impacting the quality of the optical signal. Increasing the number of channels increases the weight of the headgear and decreases its adherence to the head, making the signal more prone to movement artifacts. Therefore, choosing which cortical areas to cover implies

not measuring activity from other regions, that could also be of interest to the research question. As far as the current experiments are concerned, I was unable to look at responses from some cortical areas that were potentially interesting for affective touch processing. For instance, in Experiments 2 and 3, affective touch might have elicited stronger responses than non-affective touch in the orbitofrontal cortex, an area shown to respond to pleasant touch in adults (Francis et al., 1999, McGlone et al., 2012) and infants (Saito, 2009; Kida and Shinohara, 2013). Furthermore, in Experiments 1 and 2 I lack information from the somatosensory cortices. Future studies should aim at improving headgear designs, allowing measurements from these other regions as well to ensure a more complete understanding of these responses.

Another limitation concerns the degree of control I had over the delivery of the tactile stimulation. Notably, pressure applied through the hand during the affective touch condition might have been different from pressure applied with the spoon and with the toothbrush. Even though I strived to maintain pressure as consistently as possible across the stimuli via gentle application and by checking for deeper skin indentation as a consequence of more pressure, slight differences may have still occurred. Pressure is easy to control when applying stimulation mechanically (e.g. Löken et al., 2009; Olausson et al., 2002), but much more difficult to control when using naturalistic stimulation, such as affective touch stroking with the human hand. We note, however, that increased pressure was previously shown to elicit stronger responses in the somatosensory system, but not in areas that encoded the pleasantness of the stimuli (Francis et al., 1999).

Chapter 5

Does affective touch modulate heart rate and attention in 6 to 10-month-old infants?

Experiments 1, 2 and 3 indicated that cortical specialization in IFG and pSTS to affective touch has not yet developed in 5 to 7-months-old infants. Experiments 2 and 3 shed light on the interesting phenomenon whereby, while this cortical specialization is still underway, non-affective touch can interfere with the processing of affective touch and some paradoxical responses were observed. Specialization over pSTS could eventually be in place towards the end of the first year of life, as seems to be indicated by results from Experiment 4.

Although I observed no evidence of cortical specialization during most part of the first year of life, this does not mean that selectivity to touch could not be measured at a level other from the cortical one during early development. The fact that the differential effects of affective touch are not mediated by activation of key nodes of the social brain justifies that responses to this stimulus are investigated in other systems, such as the autonomic nervous system (ANS). Indeed, affective touch could elicit its beneficial effects via regulating activity of the two branches of the ANS.

Therefore, while in Chapter 3 and 4 I addressed the question as to whether affective touch elicits activation in social brain areas previously associated with its processing in adults, in Chapters 5 and 6 I shift the focus of attention on to a different measurement modality. In these chapters I measured heart rate changes to CT-optimal and suboptimal touch with the goal to clarify how affective touch impacts the ANS in infancy.

5.1 Introduction

The experiment (Experiment 5) in this chapter has two aims. The first aim of Experiment 5 is to measure whether affective and non-affective touch elicit differential effects on heart rate between 6 and 10 months of age. As reviewed in Chapter 1, it was proposed that social touch elicits parasympathetic activity, which manifests as decreased heart rate and blood pressure (e.g. Ditzen et al., 2007; Grewen et al., 2005; Light et al., 2005). Heart rate decelerations have been reported in response to different forms of social touch (static, affiliative and affective) in both animals and human adults. Also CT-afferent mediated affective touch was shown to elicit heart rate decelerations in adults and in 9-month-old infants (Fairhurst et al., 2014; Pawling et al., 2017a; 2017b; Triscoli

et al., 2017a; 2017b). Since to date only one paper showed that affective touch (compared to stimulation performed at CT suboptimal speeds) decreases heart rate in infants (Fairhurst et al., 2014), I set to replicate this finding. In their study, Fairhurst and colleagues applied each touch (three speeds: slow/medium-*CT-optimal* /fast) for 10 seconds. Averaging heart rate across the entire stimulus presentation they showed that affective but not non-affective touch decreased heart rate (Figure 5.2a). The timecourse of the heart rate changes from one individual infant in this study (Figure 5.2a) suggests that the decrease to affective touch is sustained throughout the presentation of the stimulus. Thus, the first hypothesis of this experiment is that affective but not non-affective touch will lead to sustained heart rate decelerations.

The second aim of this experiment is to link touch-induced heart rate changes to changes in attention. Since heart rate is an index of arousal[28], I set to test the hypothesis that touch modulates heart rate, shifting arousal to a level optimal for focused attention. The motivation for looking at this mediation model is based on the fact that a handful of longitudinal studies have suggested that touch interventions with preterm infants improved cognitive development (sections 1.2.1 and 1.2.2). Therefore, I wanted to try and understand what systems could mediate such effects. To this end I aimed to isolate the direct link between affective touch and cognition which in turn could also help to shed light on the mechanisms that mediate the longer-term effects. I chose attention as a cognitive domain for its well-known links with autonomic arousal. Given the proposed relationship between social touch (including CT-afferent mediated affective touch) and the parasympathetic system, it was reasonable to investigate if a relationship between touch, arousal and attention could be measured.

5.1.1 Arousal and the Aston Jones model of attention

For more than a century arousal has been linked to performance. The U-inverted shape function that describes this relationship (Figure 5.1a) was first proposed in the Yerkes-Dodson-Law. In one of the earliest studies, rats'

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[28] Arousal is defined as referring to the total levels of activity within the ANS.

performance in a maze was measured following electrical shocks. Mild electrical shocks improved performance leading to maze completion. However, as the stimulus intensity increased, rats started running in random directions and did not complete the maze (Yerkes & Dodson, 1908). Aston Jones built on this model advancing the idea that this curvilinear relationship is modulated by activity in the Locus Coeruleus (LC), a nucleus located in the brainstem which is part of the Reticular Activating System[29]. The Locus Coeruleus is the principal site for brain synthesis of norepinephrine (NE). NE release from the LC covaries with circadian rhythms (low firing rates are associated with sleep, high firing rates with states of wake). However, the most interesting feature of the LC is how during states of wake its activity, both tonic and phasic, modulates attentional behaviours (Figure 5.1b). Notably, low firing rates of LC neurons are observed in animals when they are engaged in automatic activities such as grooming and feeding and they are not paying attention to external stimuli. Medium LC activity is associated with states of 'selective' or 'focused' attention where the animal is not easily distracted by stimuli outside of their focal area. States of focused attention are necessary for goal-directed behaviours. High LC activity is associated with high vigilance to the surroundings. In this state, behaviour is characterised by scanning the environment, making short fixations and thresholds to respond to various features are decreased (Aston-Jones & Bloom, 1981a, 1981b; Aston-Jones, et al., 1999; Rajkowski et al., 1994). Shifting between these states allows the organism to respond to the environment in a flexible manner. For example, in novel or uncertain environments high LC activity helps to maintain a vigilant state and to be ready for fight or flight. In a safe, stable environment, the organism should maintain a level of medium arousal, optimal for assimilating food and information.

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[29] The Reticular Activating System is a set of connected nuclei in the brains of vertebrates that is responsible for regulating wakefulness and sleep-wake transitions. Besides the noradrenergic nucleus (the LC) other nuclei are dopaminergic, serotonergic, histaminergic, cholinergic, and glutamatergic. These nuclei project to the cortex either via direct axonal projections or indirect projections through the thalamus.

a)   b)
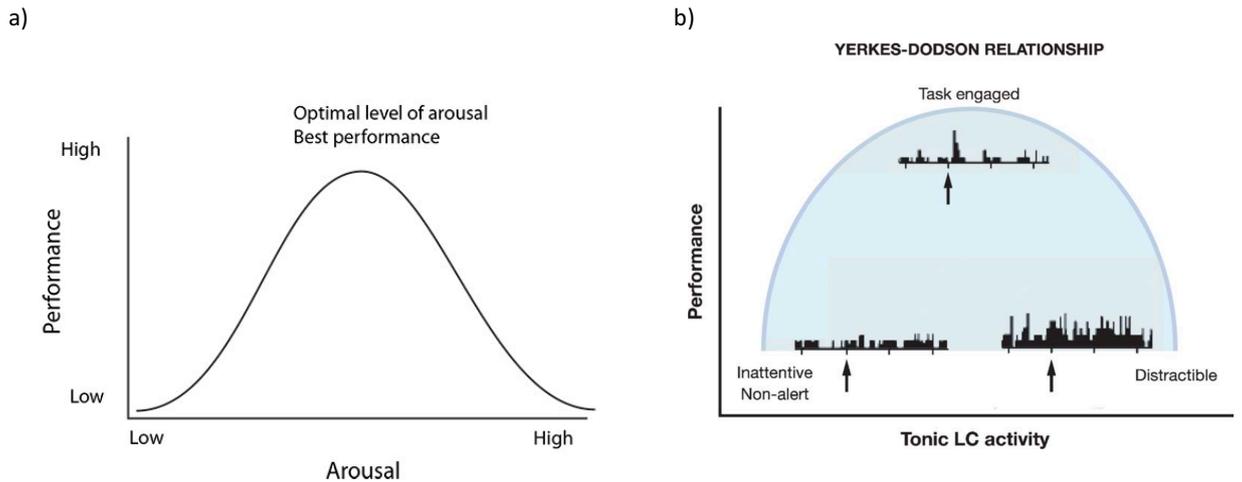
Figure 5.1 a) Yerkes-Dodson inverted-U relationship between arousal and performance. b) Inverted-U relationship between LC activity and performance on tasks that require focused attention. (Aston-Jones et al. 1999). Performance is poor at very low levels of LC tonic discharge because animals are drowsy and non-alert. Performance is optimal with moderate LC tonic activity and prominent phasic LC activation following goal-relevant stimuli (phasic LC mode). Performance is poor at high levels of tonic LC activity (tonic mode, lacking phasic LC activity). This resembles the classical Yerkes-Dodson relationship.

5.1.2 Arousal and the autonomic nervous system

Our current knowledge of the relationship between attention and the LC-NE system comes from animal studies (with rodents and non-human primates) where attentional behaviour was measured alongside single cell recordings from LC-NE neurons (Aston-Jones and Bloom, 1981a; 1981b). To test the validity of this framework in humans the first limitation that needs addressing relates to how to measure the LC-NE system activity, given that single cell recordings are not possible. A solution is offered by connections between the LC and the autonomic nervous system (ANS). Since the ANS is directly under the influence of the LC, we can assess activity of the LC-NE system via measuring changes in peripheral indices of the ANS, such as heart rate, skin conductance and pupil dilation. In animals, to the best of my knowledge, only one study validated this relationship recording concurrently LC neurons firing rates and heart rate during stressful stimuli and showing a strong positive correlation (Abercrombie and Jacobs, 1987). However, the limitation of these findings is that they revealed an association between LC activity and activation of the

sympathetic branch of the ANS. Association with the parasympathetic branch (which would manifest as decrease in LC activity accompanied by a decrease in heart rate, both relative to baseline) was not tested since this work was interested in the stress response and thus in eliciting sympathetic activation. Therefore, an association between the LC-NE system and the parasympathetic system needs to be assumed.

de Barbaro and colleagues were the first to directly test the assumptions of the Aston Jones model of attention (AJMA) with infants, relying only on behavioural measures (de Barbaro et al., 2011). They measured how 6-7 months old infants respond to salient peripheral stimuli during a semi-naturalistic paradigm measuring their attentional vigilance. In their study infants were surrounded by six monitors that would turn on and off in a quasi-randomised sequence to play a short video (the salient stimulus); only one monitor at the time would play the video. Specifically, vigilance was measured using four measures: reorientation latencies and likelihoods and duration and rate of fixation during stimulus presentation (increased vigilance would correspond to shorter latencies of reorientation, increased likelihood of reorientation and a high rate of short fixations). Given the within subject high correlation of these behaviours they created a 'vigilance index' and, in line with the Aston Jones framework, showed that the more vigilant infants are, the faster they are to reorient to the salient stimuli (vice versa, the lower the vigilance index the longer they would attend to non-salient stimuli in the environment, such as floor or walls) (de Barbaro et al., 2011).

More recently de Barbaro and colleagues built on these findings and further tested the AJMA with infants measuring the association between attention and autonomic arousal. They measured 12-months-old infants' visual attention during a 20 minutes' battery of alternating video clips and static images while recording ANS activity (HR, EDA, movement levels) (de Barbaro et al., 2017). The results show that periods of lower arousal[30], were associated with longer looks to the visual stimuli and vice versa. Furthermore, changes in arousal occurred before changes in visual attention. Showing this relationship

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[30] ECG, EDA, head velocity and peripheral accelerometry were combined to form an 'arousal composite' given the fact that previous work had shown covariation of these measures (Wass et al., 2016, 2015).

between autonomic arousal and attention further validates the applicability of the Aston Jones framework with infant samples.

Although scarce, existing evidence is encouraging and supports the possibility of using this model when studying human infants' attention. Importantly, it indicates that changes in heart rate should reliably parallel changes in attentional behavior. Thus, bringing together different research lines, with the present work I aimed to investigate 1) how affective touch modulates arousal, indexed by changes in heart rate and 2) whether changes in arousal associate to changes in attention, indexed by time to reorient to a peripheral stimulus.

Changes in heart rate were measured in response to CT-optimal touch as this stimulus was previously shown to decrease heart rate in both infants and adults (Fairhurst et al., 2014; Pawling et al., 2017a; 2017b; Triscoli et al., 2017). The three studies that investigated HR changes in response to CT-targeted touch have yielded different types of responses (and used different lengths of stimulation). In the work of Fairhurst and colleagues brush strokes were delivered on the forearm of 9-month-old infants for 10 seconds at one of three different speeds (very slow, CT-optimal, fast) and HR changes (vs. baseline) averaged across the entire period of stimulation (Fairhurst et al., 2014). In Pawling et al., brush strokes were also delivered on the forearm and on the palm of adults' participants at two speeds (CT-optimal, fast), but for 3.33 seconds (brief stimulation). The authors looked individually at the five beats following stimulation onset (each compared to baseline; Pawling et al., 2017a; see Figure 5.2b). Finally, in Triscoli et al. adult subjects were asked to stroke their partner's forearm using a naturalistic speed (no control speed used here) for 15 seconds and HR changes (vs. baseline) averaged across the entire period of stimulation (Triscoli et al., 2017). While two studies recorded sustained changes in HR to affective touch (a sustained change was over 10 seconds of stimulation; Fairhurst et al., 2014; see Figure 5.2a, and over 15 seconds of stimulation in Triscoli et al., 2017), a study with adults showed that the decrease is present shortly after the beginning of the stimulation (from the second heart beat post stimulus onset, Pawling et al., 2017a; see Figure 5.2b). As concerns non-affective touch, one study found that it did not elicit significant differences from baseline, but a trend towards increased HR emerged (Figure

5.2a). On the other hand, another study found that in the period immediately following stimulation onset both touches elicited a decrease in HR, with affective touch eliciting a larger decrease than non-affective touch (Figure 5.2b). Therefore, to better understand heart rate changes in response to touch, in the present experiment I set to investigate how the cardiac response unfolds over time. HR was averaged across 5 seconds' segments, for a total of four segments (20 seconds).
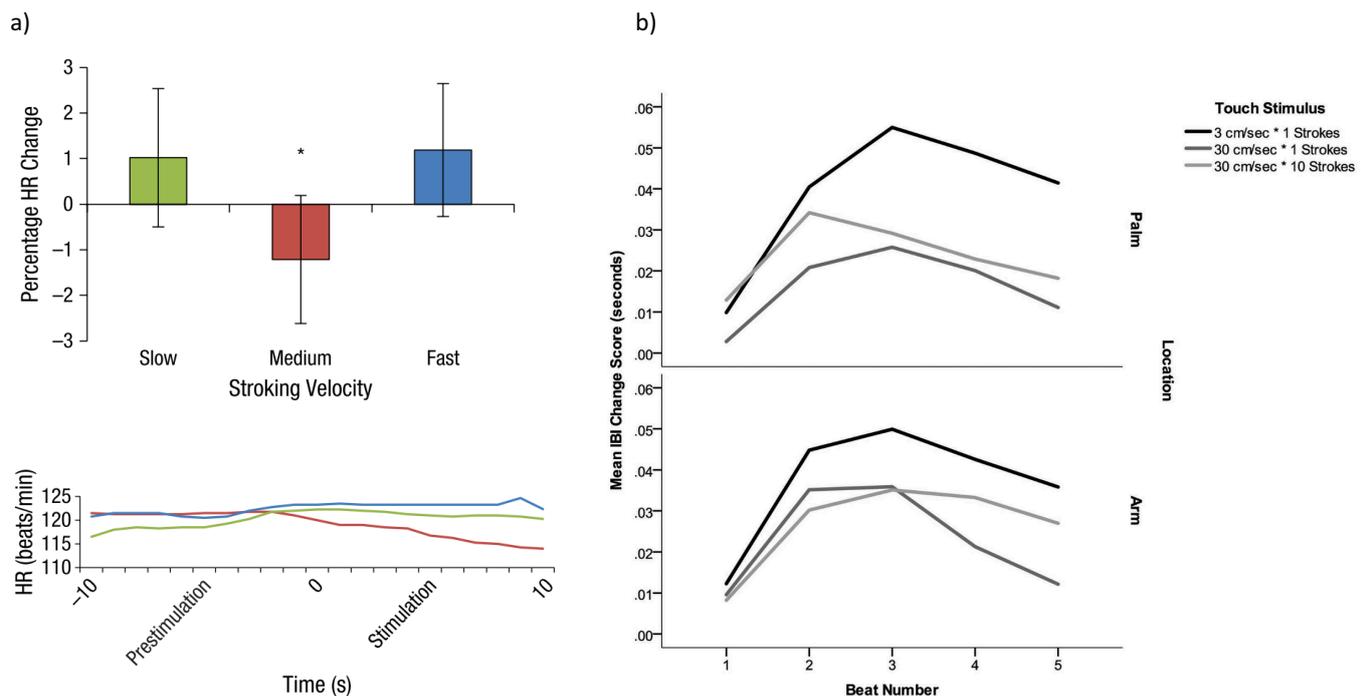
Figure 5.2 a) figure from Fairhurst et al., 2014. Top panel: mean percentage heart rate (HR) change shown as a function of stroking velocity. Bottom panel: The graph shows an individual infant's heart rate (HR) during the 10 s before stimulation and the 10 s during stimulation as a function of stroking velocity. b) figure from Pawling et al., 2017a. Heart rate responses, represented as change in IBI to CT-optimal and CT non-optimal touch stimuli, applied to the palm (top panel) and arm (bottom panel). Change scores represent change from baseline in seconds, with positive values representing longer IBIs, and thus a slowing of heart rate from baseline levels

To measure changes in attention, I looked at how long infants take to reorient from a centrally presented stimulus to a peripheral one. For this purpose, I used a modified version of the gap-overlap task (Elsabbagh et al., 2013). This task is traditionally employed to measure flexibility in attention switching and it showed to be highly sensitive to individual differences in the context of developmental disorders. Indeed, infants later diagnosed with autism spectrum disorder (ASD) show longer latencies to disengage from the central

stimulus (at 7 months: Elison & Paterson, 2013; at 12-14 months: Zwaigenbaum et al., 2005; Elsabbagh et al., 2013) when compared to both controls and infants at risk that later do not meet criteria for diagnosis. I employed this task with typically developing infants, to test if it can also be used to capture individual differences in orienting, elicited by arousal changes. From this task, I only used two of the original three conditions: Baseline (where the peripheral stimulus appears after the central stimulus disappears) and Overlap (where presentation of the peripheral stimulus overlaps with the central stimulus). Based on de Barbaro et al. (2011), I hypothesized that longer latencies to reorient to the peripheral stimulus in Baseline trials would index that the infant is at an intermediate level of arousal, which favours focused attention. Likewise, I hypothesized that longer latencies to disengage (measured as the difference between orienting in Overlap and Baseline trials, (Elsabbagh et al., 2013)), would index an optimal arousal level for focused attention.

In this study, the visual orienting task was presented in short blocks; in separate blocks infants received either no tactile stimulation (baseline condition) or tactile stimulation (slow touch at app. 5cm/s or fast touch at app. 30 cm/s). The touch type was manipulated between subjects. I set to test the following hypothesis that 1) slow touch (compared to no touch and to fast touch) will decrease heart rate and, if this decrease reflects a shift in arousal to the optimal level for focused attention, 2) infants will show i) longer latencies to reorient to the peripheral stimulus (Baseline trials) and ii) longer latencies to disengage from the central stimulus (Overlap trials).

Using the same paradigm, I tested two different age-groups: 6-7 and 8-10 months old infants. The age of the younger group matched the youngest age group with which the use Aston Jones model was validated in infants (de Barbaro et al., 2011). I also chose to test 8-10 months olds since this is the youngest age group where heart rate decreases in response to CT targeted touch were reported (Fairhurst et al., 2014). Having two age groups allows me to explore whether touch-dependent modulation of arousal changes between 6 and 10 months (is it faster in older infants?). However, I expect affective touch to elicit the same effect on attention in both age groups.

5.2 Methods

5.2.1 Participants

Thirty-two 6-month-old infants took part in the study (12 females, mean age=200.87 days, SD= 8.2, range=185-215). From this group, four infants (two in the slow touch and two in the fast touch condition) were excluded from the analysis because of insufficient data for both the eye-tracking and the ECG measures (see below for more details about inclusion criteria). Thirty-four 9-month old infants took part in the study (12 females, mean age=284.04 days, SD= 17.52, range= 251-316). Eight of these were excluded from the final analysis because of insufficient data (three in slow touch and five in fast touch condition).

Not all infants included in the final analysis contributed both eye-tracking and heart-rate data, either because of poor calibration or because of a large number of artifacts present in the ECG recording. In the younger group, 25 infants contributed data to both eye-tracking and heart-rate measures; 3 infants only contributed to the analysis with heart rate data. In the older group, 22 infants contributed data to both eye-tracking and heart-rate measures; 1 infant only contributed to the analysis with heart rate data; 3 only contributed to the analysis with eye-tracking data.

5.2.2 Stimuli and design

The visual orienting task is adapted from Elsabbagh et al. (2013). All trials began with a centrally presented animation: a clock that first expanded and then spun around (500°/sec), accompanied by an attractive sound, to engage the infant's attention. Once the infant attended to this, a peripheral target, also accompanied by a sound, appeared randomly either to the right or the left of the central animation at the eccentricity of 13°. Peripheral targets were always the same: a spinning cloud with size of 3 cm that rotated at 500°/s. The peripheral target remained displayed until the infant looked at them or until 2 seconds elapsed. Once the infant looked to the target or if the maximum duration was reached, an attractive animation of an animal with sound replaced the peripheral target for 1 second and the trial was terminated.

There were two different conditions. In the Baseline condition, the central fixation stimulus was extinguished and the peripheral target appeared simultaneously; in the Overlap condition the peripheral target appeared while the central fixation stimulus remained displayed (but not animated) so that the two stimuli overlapped. The two conditions were presented randomly within blocks of 8 trials each (4 baseline and 4 overlap trials per block). Blocks were interleaved with a 10 seconds extract from "In the night garden" showing white blooming flowers against a black background. I used this video to give infant a short break between blocks and no eye-tracking data was collected during this time. Trial presentation continued until the infant became fussy or until a maximum of 64 trials (i.e. 8 blocks) was reached.

During four of the eight blocks, infants received concurrent *tactile stimulation*. The stimulation consisted of stroking the infant's upper back, directly on the skin, using a soft paintbrush (2 cm width). Repeated stroking was applied horizontally from left to right. I used a between-subject design, with half of the infants receiving slow velocity stroking (approximately 5cm/s) and the other half fast velocity stroking (30cm/s). The first block was always a no-touch block with touch and no-touch blocks alternating thereafter (see Figure 5.3).
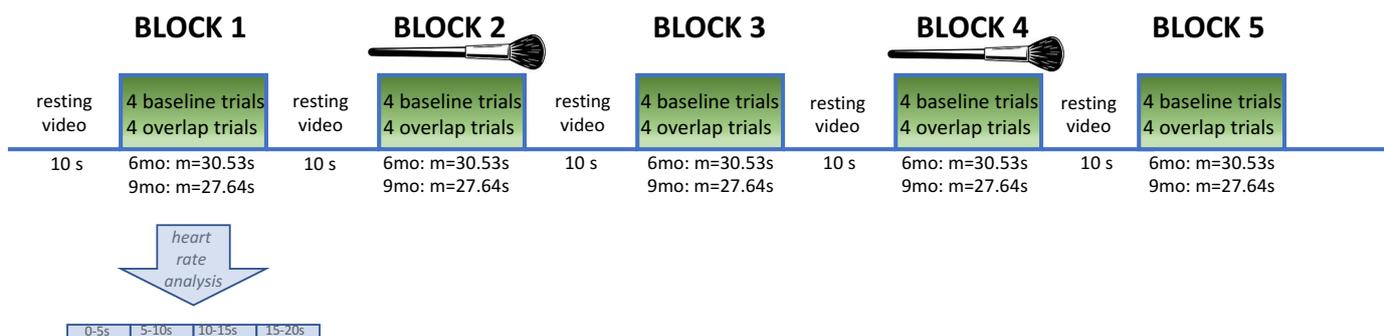
Figure 5.3 Experimental design. Each block consists of 8 trials of the visual orienting task, presented randomly. Duration of a block is variable, here mean values for each age-group are reported. A maximum of 8 blocks were presented and tactile stimulation was delivered always on blocks 2, 4, 6 and 8. Heart rate analysis were performed on the first 20 seconds of each block and IBIs averaged across 5 seconds segments.

5.2.3 Procedure

For this study, infants were changed into a sleeveless vest with a square-shaped opening on the back and ECG stickers were placed on their chest. In the eye-tracking lab, infants were seated in a Bumbo seat (secured onto a chair) 60 cm away from the eye tracker screen. The parent was asked to take a seat behind the infant's seat, making sure they remained out of the infant's sight. After ensuring correct recording of the ECG signal, the main experimenter started the eye-tracker calibration. The calibration procedure consisted of a contracting spiral that was presented at all four corners and at the centre of the screen. Once calibration was successful, the visual orienting task started.

The main experimenter then moved behind the infant and stroked their back through the opening in the vest (see Figure 5.4 for experimental set-up). A second experimenter monitored infants' looking behaviour and if the infant looked away during the task, she would play an attention-grabbing sound to redirect the infant's attention to the task. Furthermore, the second experimenter manually marked the beginning and end of each block of the visual orienting task on the ECG recording for the subsequent segmentation of the heart-rate data. The marking was checked off-line for accuracy.

Figure 5.4 Picture depicting experimental set-up.

5.2.4 Data processing, reduction and analysis

5.2.4.1 Heart rate

From the original ECG signal, a time series of inter-beat intervals was extracted for each block, for each subject. One block consists of 8 trials of visual orienting task (see Figure 5.3). Since the visual orienting task is gaze contingent, time to complete each block varied both within and between participants (6mos: m=30.53s, sd=6.76, range: 22.84-59.55s; 9mos m=27.64s, sd=4.82, range 21.46-51.56s). Therefore, to maximize the number of participants contributing data to the analysis, I only used data from the first 20 seconds of each touch/no touch block. Each 20 seconds block was then segmented into 4 five-seconds segments to obtain more detailed temporal information of the heart rate changes.

I coded motor behavior of each infant from the video recordings. Each infant was assigned one score for motor activity that ranged from 0 to 4 (0=very still, 1= little amount of movements throughout the session, 2= frequent but mild motor activity, 3= moderate motor activity, 4= pronounced motor activity). Two coders coded videos separately and Cohen's kappa showed near perfect agreement between their scores (kappa=.82, p<.001).

I excluded blocks based both on behavioural coding and on the amount of artifacts present in the signal. Following offline coding of the videos a block was excluded if during it the infant 1) cried, 2) sneezed or yawned 3) presented excessive movement that prevented the correct delivery of the stimulus. Following inspection of the inter beat intervals (IBIs) time series, a block was excluded if more than 3 consecutive beats were missing. In the younger group infants had on average 7.18 valid blocks (SD=1.15, range= 4-8); in the older group on average infants had 7.45 valid blocks (SD= 0.96, range=5-8).

I identified infant movement as a potential confounder of heart rate measurements. Indeed, movement could elicit cardiac accelerations, thus changes in heart rate would be unrelated to the tactile stimulation. However, since level of movement did not significantly correlate with heart rate (all ps>.078), I did not include this measure in further analyses.

To test the effect of condition (touch/no-touch) and of touch type on heart rate, I ran a 4x2x2x2 mixed ANOVA, with segments (0-5s, 5-10s, 10-15s,

15-20s) and condition (touch, no touch) as between subjects factors, and touch type (Slow, Fast) and age (6mo, 9mo) as within subject factors. This yielded to a main effect of condition with touch blocks eliciting larger heart rate increases than no touch blocks ($F_{(1,47)}=12.793$, $p=.012$). However, the effect of condition is contaminated by the lack of counterbalancing of conditions. I therefore investigated if the presence of a trend in the data could explain this finding.

A qualitative inspection of the raw data revealed a trend with heart rate increasing across blocks. To confirm the presence of this trend I ran a repeated measure ANOVA to test for an effect of block order. A main effect of order ($F_{(7,196)}=7.74$, $p<.001$, $\eta p^2=.209$) revealed a systematic increase in heart rate occurring throughout the session (see Figure 5.5). Touch type and age were inserted as between-subjects factors but they were not significant ($ps>.484$) indicating that this increase similarly affected all groups. Because these changes were not specific to particular blocks but reflected a linear acceleration throughout the experiment, MATLAB was used to detrend the data. For each subject a trend line (best linear fit) was calculated across the whole session. The trend line was then removed from the data. The repeated measure ANOVA run on the new detrended data yielded no main effect of block ($F_{(7,210)}=.739$, $p=.639$). Detrended data was therefore used for further analysis.

The same model as the one initially tested was run with detrended data: 4x2x2x2 mixed ANOVA, with segments (0-5s, 5-10s, 10-15s, 15-20s) and condition (touch, no touch) as between subjects factors, and touch type (Slow, Fast) and age (6mo, 9mo) as between subject factors.
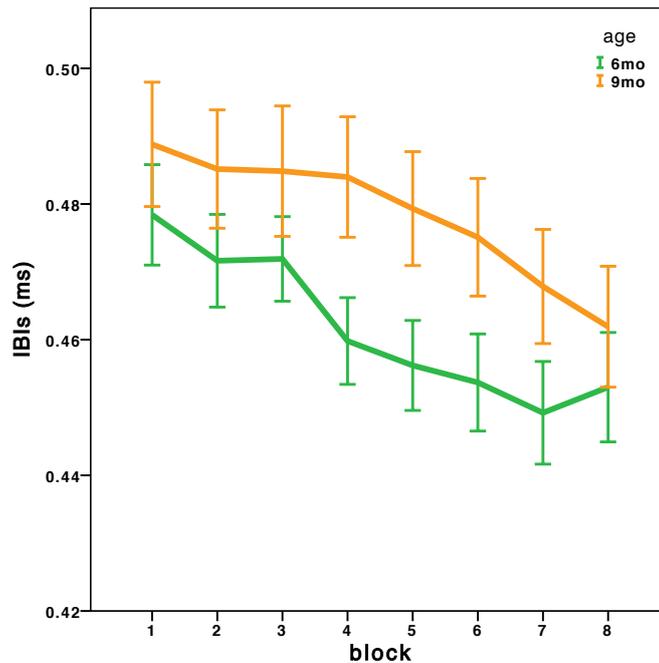
Figure 5.5 IBIs across 8 blocks displayed separately for the 6mos (green) and the 9mos (orange) groups. Error bars represent standard error.

5.2.4.2 Visual orienting reaction times

For each eye-tracking session, MATLAB programs were used to calculate reaction times to reorient to the peripheral target in each trial.

Individual reaction times were judged against two inclusion criteria. A trial in either condition (baseline or overlap) was rejected if: 1) the infant did not look at the central stimulus for at least 75% of the time that elapsed between its onset and the onset of the peripheral stimulus and 2) the infant did not orient towards the peripheral stimulus between 200 and 1200 ms after its onset. Trials that met inclusion criteria were included in further analysis. A minimum of 20 valid trials per condition was necessary in order to include an infant in the analysis. Details of valid trials per condition per group can be found in Table 5.1.

For each subject I calculated a *reorienting score* by averaging together reaction times in baseline conditions in each block, thus obtaining a maximum of eight values per subject. I looked at baseline trials because I was interested in how changes in arousal can modulate orienting to the peripheral stimulus. For each subject I also calculated a *disengagement score*. For each block, I

subtracted the average of the baseline trials from the average of the overlap trials. These scores were tested for normality using the Shapiro-Wilk test and I found that reorienting scores in 3 out of 8 blocks were not normally distributed and disengagement scores were not normally distributed in 1 block.

Since I did not counterbalance the touch/no-touch conditions and used a fixed order with no touch blocks always at the beginning, I first checked whether trial order affected the measures of interest. To this end, I ran a Generalized Estimating Equation (GEE) model, built with a linear distribution, an unstructured correlation matrix and a robust estimator for both the reorienting scores and the disengagement scores. The GEE was used to account for the non-normal distribution of the variables and for missing values (Liang & Zeger, 1986; (Pickles, 1998)).

To check for trends in each age-group, the GEE model was run separately for the 6 and the 9 months olds. The factor 'block' was entered on eight levels.

Reorienting scores. The effect of 'block' on RTs was significant for 6 mos ($\chi 2(7)$ =92.332, p<.001), as well as for 9 mos ($\chi 2(7)$=28.798, p < .001). In 6 mos, post-hoc pairwise comparisons showed that the RTs in the first three blocks were slower compared to the remaining 5 blocks (p*s*<.047), see Figure 5.6, left panel. In 9 mos, post-hoc pairwise comparisons showed that the RTs in the first block were significantly slower compared to the remaining 5 (p*s* <.024), see Figure 5.6. After removing the first block the factor 'block' was no longer significant for 9mos ($\chi 2(6)$ =4.18, p=.652). Taking into account these effects, I did not average scores across blocks of touch and no-touch separately.

Disengagement Scores. No effects of 'block' were found in either age group (Figure 5.6, right panel). Therefore, I averaged together all touch and no-touch blocks to obtain two values for each participant: 'disengagement touch' and 'disengagement no-touch'.

Normal distribution was checked within each age group for 'disengagement touch', 'disengagement no-touch' and for the reorienting scores over 8 blocks. Four younger infants, 'disengagement touch' and three of the reorienting blocks not normally distributed. For older infants, one of the reorienting blocks was not normally distributed. Thus, for subsequent analysis I used the GEE (built with a linear distribution, an unstructured correlation

matrix and a robust estimator). For disengagement scores, I tested for effects of condition (touch vs. no touch), touch type (slow vs. fast) and age (6 vs. 9 mo) using the GEE. For reorienting scores I tested for effects of condition (touch vs. no touch), block number, touch type (slow vs. fast) and age (6 vs. 9 mo) using the GEE.

		6-months-old					9-months-old				
		total valid trials	valid trials baseline	valid trials overlap	RT baseline	RT overlap	total trials	valid trials baseline	valid trials overlap	RT baseline	RT overlap
Fast Touch group	mean	49.77	26.15	23.62	398.65	514.40	53.21	27.00	26.21	390.41	476.22
	sd	6.82	3.53	4.79	32.86	84.82	9.97	5.13	5.38	50.04	66.47
Slow Touch group	mean	48.83	25.58	23.25	412.27	527.06	48.00	24.33	23.67	382.24	466.53
	sd	15.03	6.76	8.55	44.59	84.44	12.58	6.91	6.04	39.66	83.02

Table 5.1 Valid trials (total and split by condition) and reaction times (ms) in each condition. Values are split by agegroup and by touch group.
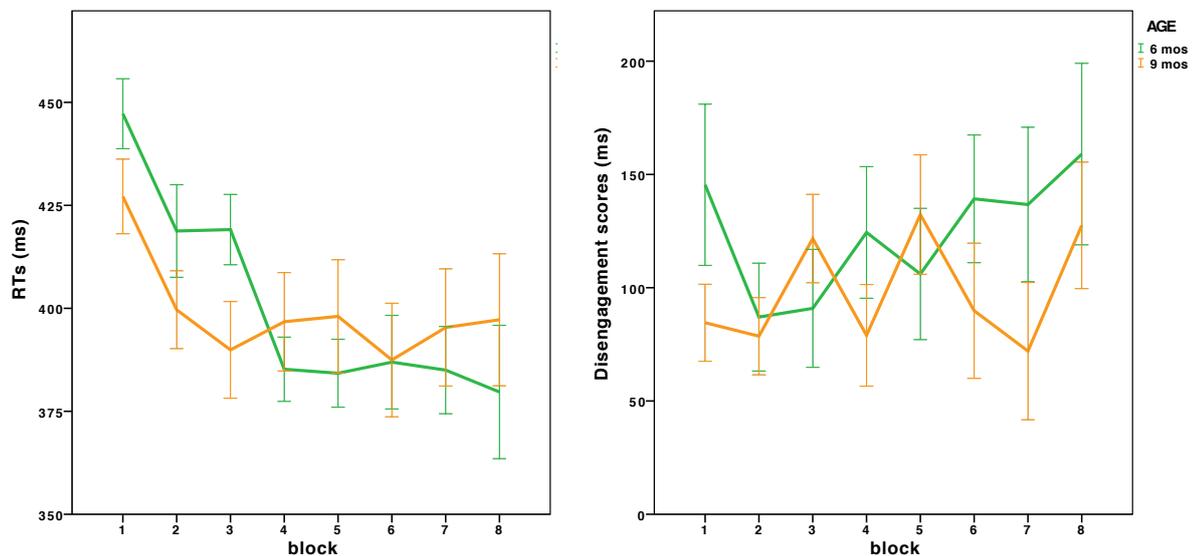
Figure 5.6 Change in RTs across 8 blocks for Reorienting scores (left panel) and disengagement scores (right panel). RTs and reorienting scores are displayed separately for 6mos (green) and for 9mos (orange). Error bars represent standard error

5.3 Results

5.3.1 Heart rate

The 4x2x2x2 (segment, condition, touch type, age) mixed ANOVA yielded a main effect of segment ($F_{(3,138)}=8.164$, p=.001, $\eta p^2=$ .151), a significant interaction between segment and age ($F_{(3,138)}=3.009$, p=.032, $\eta p^2=.061$) and a significant interaction between segment and condition ($F_{(3,118)}=3.92$, p=.017, $\eta p^2=$ .079). I found no main effect of age ($F_{(1,46)}=1.94$, p=.170). There was no main effect of touch type ($F_{(1,46)}=.094$, p=.761) and no significant interactions with touch type (ps>.511).

The interaction between segment and age is due to the fact that older infants show a marked heart rate decrease at the block onset, while younger infants show an increase (see Figure 5.7, top panel). Post hoc t-tests confirmed that the two groups differed in their response only during the first 5 seconds of stimulation. Indeed in this segment 9mos respond with a decrease compared to 6mos who show instead an increase in heart rate ($t_{(48)}=2.299$, p=0.26, d=.065).

The interaction between segment and condition was followed up with four paired-samples t-tests. Post hoc t-tests revealed significant differences in two segments. In segment 2 (5-10s) there is a larger heart rate decrease in the no-touch compared to the touch condition ($t_{(49)}=2.039$, p=.047, d=0.288). In segment 4 (15-20s) there is a larger heart rate increase in the no-touch compared to the touch condition ($t_{(49)}=-2.082$, p=.043, d=(.29) (See Figure 5.7b, bottom panel). While significant, these contrasts do not survive Bonferroni correction as these p values are above the threshold of .00125.
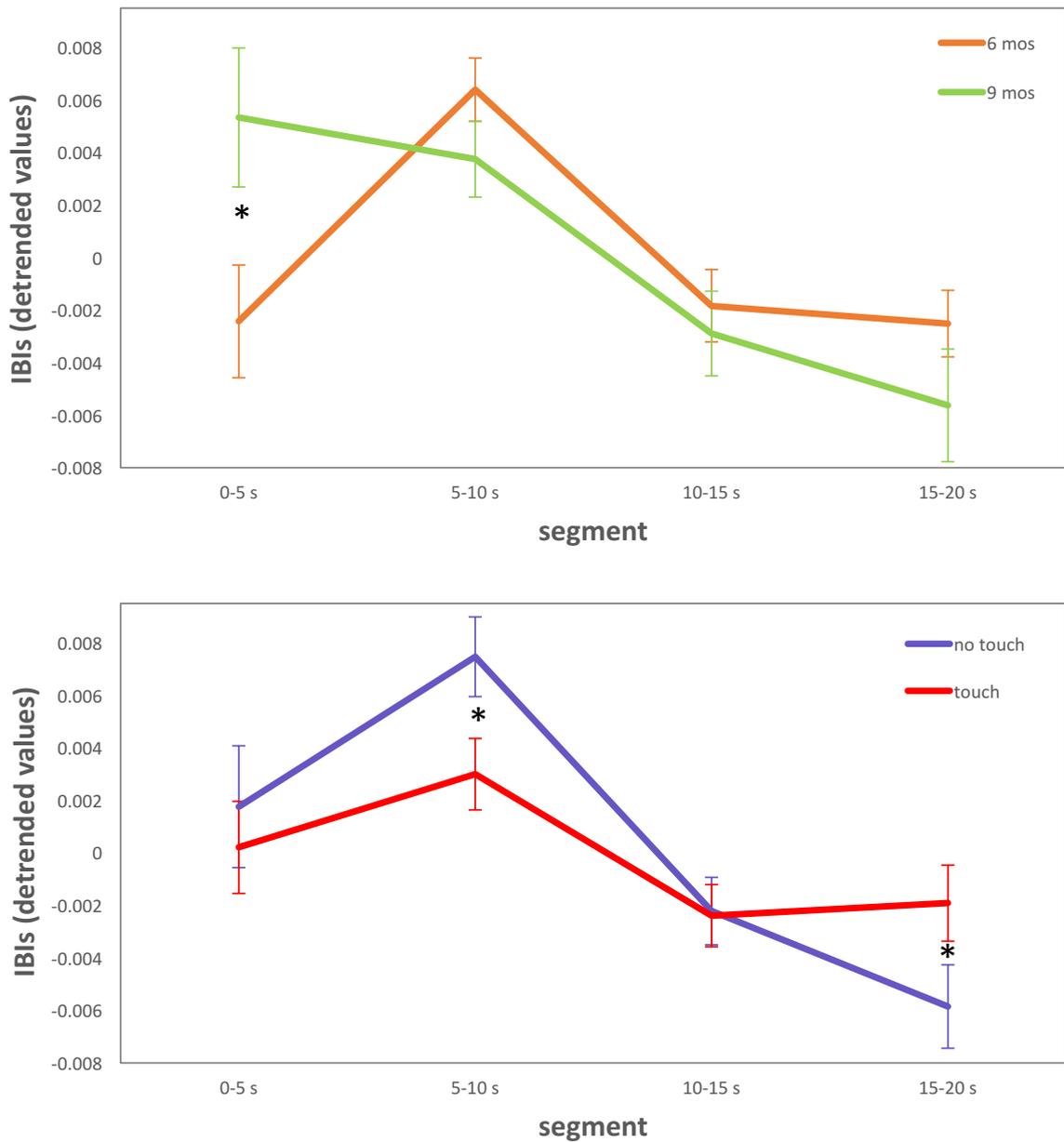
Figure 5.7 Top panel: interaction segment * age (orange=6mos; green=9mos). Bottom panel: interaction segment *condition (purple=no touch; red=touch). Asterisks indicate a significant difference at the uncorrected .05 level. Error bars represent standard error

5.3.2 Reaction times

Reorienting score.

The 2x4x2x2 condition (touch vs. no touch), block number, touch type (slow vs. fast) and age (6 vs. 9 mo) GEE showed main effect of block ($\chi 2(6)$=82.826, p < .001, main effect of condition ($\chi 2(1)$=32.976, p = .002) (with slower reorienting for the touch than no-touch condition), and a significant interaction block*age ($\chi 2(6)$=15.478, p = .017). However, the main effect of condition is driven by the

fact that in both age groups reorienting is significantly slower on the first block (always a touch block). The interaction block*age reflects the above reported finding (section 5.2.4.2) that younger infants are slower in the first 3 blocks while older infants only in the first one. Because the order of conditions was not counterbalanced, and I find a main effect of block -limited to the initial blocks- the effect of condition is not reliable, and I cannot draw conclusions about differences here. It is nonetheless worth to highlight that no effect of touch type was revealed by these analyses.

Disengagement score. The 2x2x2 condition (touch vs. no touch), touch type (slow vs. fast) and age (6 vs. 9 mo) GEE yielded no significant main effects (all p*s*>.209) nor interactions (all p*s*>.214) (see Figure 5.8, right panel).

These results suggest that receiving slow or fast touch concurrently to the visual orienting task does not affect the subject's performance.
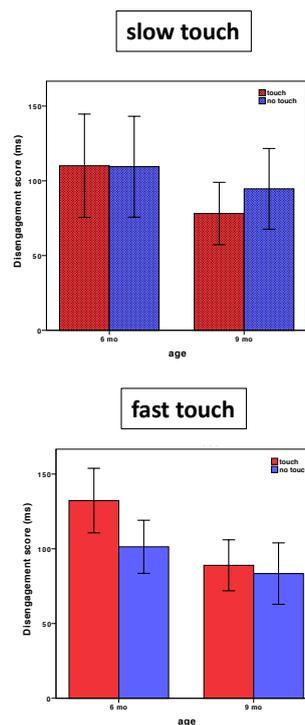
Figure 5.8 Disengagement scores split by age, touch condition (red=touch; purple=no touch) and touch type (patterned bars=slow touch; plain bars=fast touch). Error bars represent standard error

5.4 Discussion

In this experiment I aimed to test the hypotheses that affective (vs. non-affective) touch would lead to a sustained decrease in heart rate and to longer latencies to reorient to a peripheral stimulus in a visual orienting task. The results support neither hypothesis. Notably, affective and non-affective touch do not elicit differential responses on either heart rate or on reaction times.

As concerns heart rate, based on findings in adults (Pawling et al., 2017a; 2017b) I expected a larger decrease to affective touch compared to both non-affective touch and no touch in the first segment post stimulus onset (0-5s), but it did not emerge from this study. In the present experiment, touch decreases heart rate between 5-10s, in line with the sustained response reported by Fairhurst et al. (2014) to a 10s stimulation. However, this decrease is not specific to affective touch and it is smaller when compared to the decrease observed in the same segment in the absence of touch.

Indeed, while I do not observe a difference between affective and non-affective touch, the findings reveal differences between touch and no-touch blocks. In the absence of touch, I observed a larger decrease 5-10s post stimulus onset and a larger increase 15-20s post stimulus onset compared to the blocks with touch. Thus, being stroked seems to modulate the cardiac response elicited by the task, independently of the speed of the stroking. More precisely, touch decreases the variation in heart rate during the 20 seconds of stimulation (i.e. a flatter response). It would seem that when the visual attention task and the tactile stimulus co-occur, the autonomic response is dampened possibly signaling competition between the stimuli. It is interesting that touch seems to elicit this effect in general, regardless of it being affective or non-affective.

One possibility is that the nature of the visual task affected how touch modulated heart rate. Although visual stimulation was present in Fairhurst's experimental setting as well, in their study infants watched a silent cartoon video from which they could withdraw their gaze (without causing the video to stop). In contrast, in the present experiment instead of "free viewing" scenes from a cartoon, infants followed a sequence of animations, each associated to an attractive sound, presented on monochrome background, which paused while the infant looked away. Thus, in the visual orienting task, the sequence of

central and peripheral animations (only elements on the screen) is what drives the infants' attention. Whereas during free viewing of a video, infants choose to which element of the scene to allocate their attention. Therefore, differences in how the two visual stimulations engaged attention in the two studies could explain the differences in heart rate responses.

During free viewing of a video, Fairhurst et al. measured the expected effect of touch on heart rate, while with the present experimental design I could not replicate their finding. Could this suggest that as soon as the infant attention is actively engaged by something more salient than the touch itself, affective touch ceases to elicit this effect? While in Fairhurst the tactile stimulation could have been the most salient element, in this study the same stimulation might have had lower saliency because 1) the infant could not see it, and 2) it was presented concurrently to a stimulation that engaged the infants' attention in a controlled way.

While we can observe how the autonomic response to the task is modulated by presence of touch, we cannot evaluate the opposite (i.e. how the autonomic response to the touch is modulated by the task). Presumably, in a touch only condition (without the co-occurrence of the visual task) we would not observe a cardiac acceleration, given that touch (not only affective) has been usually associated with heart rate decreases (e.g. Pawling et al., 2017a, 2017b; Triscoli et al., 2017). The increase in heart rate that occurs 10-20s post stimulus onset could be elicited by the task execution. Mental effort has been associated with increased autonomic activity, including heart rate, in adults (e.g. Mulder et al., 1995).

Alternatively, the visual attention task could be itself arousing due to the animated stimuli paired with attractive sounds, and the heart rate increase that we observe 10-20s post stimulus onset, could instead reflect the use of engaging stimuli rather than mental effort. If this is the case, the dampened cardiac acceleration observed in presence of touch could reflect an increase in parasympathetic activity (elicited by the touch) competing with sympathetic activity (elicited by the task). The possibility that the physical properties of the stimuli impacted the response could be tested by running the same experiment with continuous background music (i.e. the one used in Experiments 1 and 2) or using static images, instead of animated ones; such changes would certainly

reduce the attractiveness of the stimuli and possibly make them less arousing. Stimuli for this task were originally chosen to engage the infant as much as possible in order to maximize the number of completed trials, without considering the impact on autonomic arousal.

Heart rate data across the entire experimental session (before averaging the blocks) presented a linear increase (which was removed with detrending). Could this reflect a linear increase in mental effort throughout blocks of the task? Or else, could this be explained by the fact that the task stimuli incrementally increase arousal? If running the experiment with less engaging stimuli also rids of this trend in the data, then we could claim that the stimuli caused the linear increase. Else, should this trend persist, it would be linked to the condition of being engaged in the visual attention task.

Returning to my original hypotheses, the first one predicted that affective touch would have led to a cardiac deceleration compared to non-affective touch. This hypothesis was not confirmed. The hypothesis was based on the only study that showed this effect in infants (Fairhurst et al., 2014). The possibility exists that this result was a false positive as no replication of this study has yet been published. As it emerged from the results, it is possible that the visual orienting task might have interfered with the responses to touch. Therefore, from this experiment I cannot firmly conclude that affective touch does not decelerate heart rate in the two age groups tested. As concerns heart rate, I must limit my conclusion to saying that in presence of the visual task employed in this experiment, affective touch does not decrease heart rate. However, the fact that I did not measure the expected response to affective touch using this experimental design sheds light on the specificity of this response. Clearly these findings suggest that affective touch does not decrease heart rate in any circumstance, and that the effects of touch might be contextual and perhaps limited to particular initial levels of arousal.

The second hypothesis, relative to attention, built on the first one. Indeed, I expected that if affective touch decreased arousal this decrease would have reflected in longer latencies to reorient to the periphery and to disengage from the central stimulus. According to heart rate findings, I did not meet the aim to decrease arousal. However, a decrease could have been present in other arousal indexes not measured in this experiment. Thus, the present results do

not support the statement that arousal was not decreased. Even if a dampening of the heart rate response was observed (not specific to touch type), it did not lead to any differences in reaction times. Therefore, if touch actually modulated arousal, this was not sufficient to measure any changes in reorienting or disengagement in the visual orienting task. Indeed, reorienting and disengagement scores did not differ across touch and no-touch blocks (the main effect of condition observed for the reorienting score was confounded by the main effect of block therefore preventing any conclusions on a difference between conditions).

One possibility is that arousal modulation of attention is slow and perhaps occurs over a longer timescale than that assessed in the current experiment. Thus, in order to observe the hypothesized effects on attention mediated by arousal, the tactile stimulation might have to start earlier relative to the onset of the visual attention task. In a study of adults, autonomic arousal was manipulated via showing subjects videos depicting natural or urban scenes prior to assessing performance the Posner's attention task (Laumann et al., 2003). The authors show that viewing natural scenes decreases heart rate and task performance differs between the two groups (decreased arousal leads no difference in reaction times to validly and invalidly cued trials). A new experiment where touch is delivered prior to the task and not during it would also have the advantage to show the effects on heart rate specific to touch which might have been hindered by the current experimental design. Such an experiment would involve a between-subjects design with three groups: affective touch, non-affective touch, no touch. However, before persisting to test together the two hypotheses presented in this chapter, I wanted to first replicate the (hitherto isolated) finding that in infants affective (vs. non-affective) touch decreases heart rate within subjects. Therefore, I decided to take a step back and focus only on the first hypothesis of the current experiment, leaving out the link with visual attention for the time being. In the following Chapter I went on to test whether affective and non-affective touch elicit differential changes in heart rate using a within subject design. The major change between Experiment 5 and 6 is the age group: in Experiment 6 I tested 1 to 3-month-old infants. The possibility exists that findings from Fairhurst and colleagues were a false positive and that, according to the present experiment,

affective touch does not elicit sustained cardiac decelerations between 6 and 10 months. Should this be the case an explanation for my findings (lack of differential response) could lie in the age group I tested.

Using parent-child interactions, it was suggested that between 1 and 8 months there is a decrease of use of affectionate touch (Jean et al., 2009, Crnic et al., 1983). Therefore, it is possible that infants that took part in Experiment 5 are not frequently exposed to affective touch, and perhaps at this age a different touch type is used to regulate arousal. Maybe it is more likely that I will observe the hypothesised effects on heart rate at an age when affective touch is frequent compared to an age when its presence is more sporadic.

Chapter 6

Can affective touch modulate heart rate

in 1-3 month old infants?

6.1 Introduction

In the current chapter I continue testing the same hypothesis as in Chapter 5 (does affective touch decrease heart rate?) but address it employing a new experimental design. For the reasons highlighted in the discussion of Chapter 5, the experimental design employed in Experiment 5 could have hindered the response I set out to measure. Therefore, the present experiment (Experiment 6) aims to overcome the potential limitations of the previous one and clarify whether and how heart rate is modulated by affective and non-affective touch in infants. Further to the change in experimental design, the other fundamental difference between experiments 5 and 6 is the age of the infants tested. Experiment 6 was run with 1-3 month old infants. This is because a lack of a differential response in Chapter 5 might be due the documented decrease in the use of touch in parent child interaction from the first postnatal months to 6-10 months (Jean et al., 2009, Crnic, 1983). Is perhaps affective touch more abundant during the first months of life because this is the time when it is more important for development? If the effects of affective touch on development are specific to the first few months of life, then perhaps it is during that time that differential effects on the organism can be observed. Maybe if there is a time-window for affective touch to elicit its effects (as it is the case for rodents, Champagne et al., 2003; see Chapter 1), testing infants outside of this hypothetical time-window could explain why I failed to observe the predicted effects.

Changes in experimental design compared to Experiment 5 reflect the intention to more closely resemble the experimental designs employed with infants (Fairhurst et al., 2014) and adults (Pawling et al., 2017a), with which a differential response to affective touch was observed. Indeed, in Experiment 6 each infant receives both affective and non-affective touch (as in both Fairhurst's and Pawling's works) while viewing a video depicting moving shapes accompanied by music (our video differed from the one employed in Fairhurst et al. but free viewing of the video was common to both experiments). Besides similarities, two elements set apart the current study from Fairhurst's experiment. The first is that infants in our setting have no visual access to the tactile stimulus or to the experimenter. The reason for decontextualizing the

tactile stimulation is to tease apart the impact on heart rate specific to affective touch. In their work, Fairhurst and colleagues had observed that infants looked longer at the paintbrush used for stimulation during affective versus non-affective touch, thus the response they measure could have been mediated by visual attention. By preventing the infant visual access to the stimulation, I aim to clarify this finding. The second difference is the age group: 1-3 vs. 9-month-olds in Fairhurst's work. The choice of this age-group, as mentioned above, follows from 1) work in rodents that clearly indicated a critical period (the first postnatal week) for licking and grooming to have short- and long-term effects (Champagne et al., 2003) and 2) observational work that captured a decrease in parental touch during the first moths of life (Jean et al., 2009, Crnic et l., 1983). If affective touch is as critical for human development so early in life as licking and grooming is for rodents, it should impact the organism distinctly already at this age and in absence of contextual cues. Therefore, the recording of differential responses would reflect that, at the autonomic level, infants this age are already sensitive to affective touch.

In this study I contrasted responses to affective and non-affective touch, compared to a no tactile stimulation baseline. As in Experiment 5, affective touch consisted in slow brush stroking whereas non-affective touch was applied as fast-brush stroking. In line with the previous literature (and with the hypothesis of Experiment 5), I hypothesise that affective touch will decrease heart rate in the sample tested. Also in this experiment I took the approach of investigating the time-course of the cardiac responses to touch by looking separately at early (0-5s) and sustained (5-10s) responses. The expectation for a larger decrease to affective vs. non-affective touch in the early time-window is motivated by the fact that this effect was reported in adults (Pawling et al., 2017a). I then predict that this initial larger decrease to remain sustained throughout stimulation, and thus observe it also in the second time window, based on findings with 9-months-old infants (Fairhurst et al., 2014).

A sustained deceleration in HR could be subject to a double interpretation. In line with adult's work and findings in animal, it could reflect a 'calming response' elicited by parasympathetic dominance, as the one observed in response to maternal carrying (Esposito et al., 2013). Alternatively it could also index that the infant has entered a state of sustained attention, during

which heart rate deceleration is a consequence of an increase in general arousal (Richards, 2001). Since it would not be possible to draw firm conclusions further research would be needed to support either interpretation. Studies of sustained attention have typically employed audiovisual stimuli and never looked at touch, offering no points of comparison.

Besides directly measuring heart rate responses to touch, another aim of this experiment was that of exploring the potential role of two factors in influencing this response: infant's direct experience of affective touch and parental attitude to social touch. I was interested to investigate to what degree the variability in the amount of affective touch experienced by different infants is related to their physiological response to affective touch. Do those infants that are exposed to affective touch more frequently in daily life also show larger heart rate decreases to the CT-afferent mediated affective touch employed here? Such questions are inevitably linked to the one regarding the variability of this behavior during caregiver-infant interactions. How often do parents employ CT-optimal touch (gentle stroking) when interacting with their infants? To this end, at the time of the visit, mothers were asked to fill in a brief self-report measure devised to capture frequency of infant stroking (from Sharp et al., 2012).

Further to the role of experience I tested whether parental attitude to social touch relates to infants' responses to affective touch. If infants of parents which enjoy the giving, receiving and witnessing of social touch show larger cardiac decelerations to affective touch, it can be suggested that sensitivity to affective touch is heritable. An interesting finding from Fairhurst and colleagues is that 9 month-old infants whose mothers had a better attitude to social touch showed the largest heart rate decreases to CT-optimal touch (Fairhurst et al., 2014). Initially I was interested in attempting to replicate this finding in our sample as well. However, having this measure from one parent only does not strongly support genetic influences on sensitivity to affective touch. Indeed, it could be possible that mothers who have a better attitude to social touch also use more affective touch with their infants and thus sensitivity to touch could be mediated by experience rather than inherited. To better address the heritability hypothesis in this study, the attitude to social touch was measured

from both parents.[31] I hypothesise that if sensitivity to affective touch is experience dependent, higher frequencies of maternal stroking will be associated to the magnitude of the heart rate decrease. Otherwise if sensitivity to affective touch is heritable, the magnitude of the cardiac response will be associated to the parental attitude to touch regardless of amount of touch directly experienced.

6.2 Methods

6.2.1 Participants

Fifty-four 1-3-month-old infants participated in this study (25 female, mean age = 71.2 days, SD = 22.69, range=34-110 days). A further 5 infants participated but were excluded from the study because of an insufficient number of valid trials. All infants were born full term (37–42 weeks' gestation) and with normal birth weight (>2500 g). All parents gave written informed consent before the study and the ethics committee at Birkbeck, University of London, approved the study design.

6.2.2 Stimuli and design

The stimulation consisted of stroking the infant's right lower leg using a soft paintbrush (2 cm width) using one of two velocities (5cm/s or 30 cm/s). Repeated stroking was applied from the knee to the ankle. Each experimental trial was 10 seconds long (Figure 6.1a). To time the presentation of the stimuli, audio cues were played to indicate the beginning and the end of each trial. Given that the lower leg of the infants in our sample had a length of approximately 10cm, slow stroking trials consisted on average of 5 strokes (1 stroke every two seconds), while fast stroking ones consisted on average of 30 strokes.

Since infants' unpredictable movements can induce alterations to this speed (if they move their leg during stimulation) the stroke could vary in speed. If the experimenter was halfway through a stroke when the end of the trial was

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[31] Of course it does not rule out the possibility that both parents use more affective touch with their infants.

signaled she would complete it, which could add an additional one-two seconds to the duration of the stimulation. Following each 10 seconds trial there was a period of no-touch baseline, which lasted 20 seconds. All participants received stimulation on the right leg. This location was chosen because it allowed the experimenter to deliver the touch without being seen by the infant. As it can be seen in fig. 6.1, the left side of the car seat leans against the back of the seat onto which the car seat is secured; this arrangement due to this arrangement only the right side of the infant's body was accessible for stimulation. The order of presentation of the stimuli (slow/fast) was counterbalanced across participants, with half of the participants receiving the slow stimulation on the first trial, and half the fast stimulation. The order of the stimuli was pseudorandom (ABBA BAAB ABAB BAAB). During the procedure participants watched a colorful screensaver accompanied by music (the same used for the experiments in Chapters 3 and 4), to prevent them from attending to the tactile stimulation.

a)
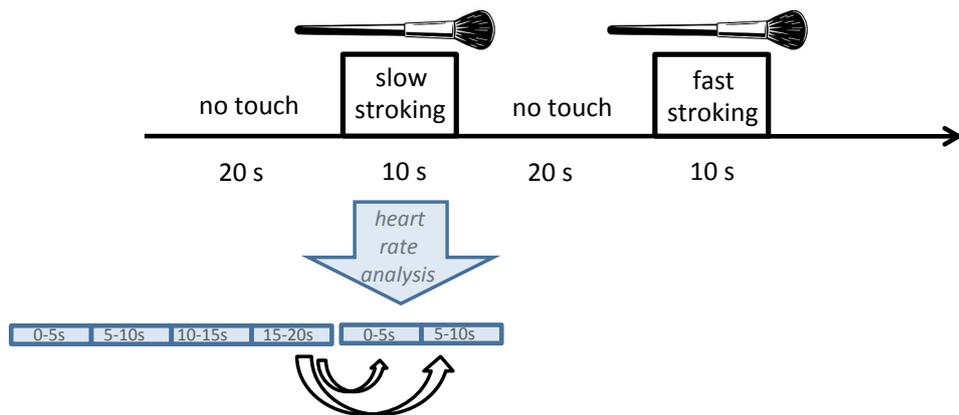
b)

Figure 6.1 a) Experimental design: the stroking was performed using a soft brush; experimental trials were 10 seconds long interleaved with baseline periods of 20 seconds. IBIs are averaged across 5 seconds segments and the last segment of the baseline is used to baseline correct each segment of the experimental trials. b) Experimental setup. The infant was seated in an infants' car seat in front of a tilted TV-screen. The yellow arrow indicates both the portion of the leg where stroking is applied and the direction of each stroke

6.2.3 Procedure

Prior to the beginning of the experiment, we laid the infant down on a car seat secured onto a chair. The infant faced a 23'' screen tilted to ensure that his/her eyes were directed at the centre of the screen (see Figure 6.1b). We chose to use the car seat in order to avoid any other form of tactile interaction with the infant besides the touch delivered by the experimenter. The parent was asked

to sit behind the infant, and refrain from interacting during the experiment unless the infant became fussy or sought their attention. We allowed a few minutes for the infant to familiarise with this novel context and began the experiment once he/she was calm and engaged with the screensaver. The experimenter sat next to the infant and delivered the tactile stimulations on the baby's right leg, being careful to remain out of the baby's sight. Events (trial onset and offset) were marked on-line by a second experimenter cued by the same audio cues used by the main experimenter to begin and end each stimulus presentation. The experiment ended when the infants became fussy. Each session was recorded using a video camera placed just above the screen, and infant behaviour was coded offline.

n	54
age (days)	71.2 (22.69), median: 70.5
female/male	25:29
number of trials completed	13.59 (3.29)
valid trials	12.57 (3.61), range: 4-17
valid trials in affective touch condition	6.46(2.006)
valid trials in non-affective touch condition	6.11(1.79)
movement score	2.078 (1.09)

Table 6.1 Descriptive values are expressed as means and standard deviations.

6.2.4 Physiological and self-report measures

6.2.4.1 Heart rate

Heart rate in this experiment was measured using ECG as described in detail in Chapter 2. Upon arrival the infant was changed into a sleeveless bodysuit and the three ECG stickers were placed on their chest and connected to the lead wires. Once in the car seat the wires were attached to the monitor, which was placed next to the infant out of their reach.

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6.2.4.2 Questionnaires

I used two self-report measures to assess frequency of maternal stroking and parental attitude to social touch. The Parent-Infant Caregiving Scale (PICS; Sharp et al., 2012) consists of 12 items where mothers have to report on how often (1=never, 2=rarely, 3=sometimes, 4=often, 5=a lot) they engage in certain behaviours with their babies (e.g.. talking, holding, kissing). Four items ask how frequently they stroked their baby's face, back, tummy, arms and legs; scores to these four items were averaged together into a total stroking score. Internal consistency (Chronbach's Alpha) for the 12 items of the PICS was .780.

The second measure used is a modified version of the Social Touch Questionnaire (STQ) with 17 of the original 20 items (Wilhelm et al., 2001). This questionnaire assesses the subject's attitude towards social touch. On a 0–4 scale (0=not at all, 1=slightly, 2=moderately, 3=very, 4=extremely) subjects have to "indicate how characteristic or true each of the following statements is of you". Positive items such as "I generally seek physical contact with others " were reverse scored. Scores range from 0 to 67; higher scores indicate an aversion to giving, receiving and witnessing social touch while lower scores indicate a greater preference for social touch. Internal consistency for the 17 items was .823. Primary caregivers were asked to share with their partners (if not present during the visit) a link to an online version of this questionnaire. Internal consistency for scores collected from second parents was .854.

6.2.5 Data processing and analysis

I excluded experimental and baseline trials based both on behavioural coding and on the amount of artefacts present in the signal. Following offline coding of the videos a trial was excluded if during it the infant 1) cried, 2) sneezed or yawned 3) presented excessive movement that prevented the correct delivery of the stimulus. Following visual inspection of the ECG tracing a trial was excluded if more than 3 consecutive beats were missing. If a baseline trial was rejected owing to any of these criteria I also rejected the following experimental trial. Number of valid experimental trials in each condition is reported in Table 1.

In addition, for the remaining trials, I coded motor behavior of each infant from the video recordings. Each infant was assigned one score for motor activity that ranged from 0 to 4 (0=very still, 1= little amount of movements throughout the session, 2= frequent but mild motor activity, 3= moderate motor activity, 4= pronounced motor activity). Two coders coded videos separately and Cohen's kappa showed near perfect agreement between their scores (kappa=.82, p<.001). This measure was used as a covariate in the analysis.

Following the preprocessing steps delineated in Chapter 2, I calculated IBIs and segmented experimental trials in two-5-seconds segments for each experimental trial (0-5s, 5-10s). To baseline correct I subtracted the average of the last 5-seconds-segment of the baseline prior to the stimulation to each segment of the following stimulation.

6.2.5.1 Preliminary analysis for potential confounders

I identified two potential confounders of HR measurements: infant movement and the number of trials contributed to analysis (See table 1 with descriptives). Movement could elicit cardiac accelerations, thus changes in heart rate would be unrelated to the tactile stimulation. On the other hand, the number of valid trials could confound the heart rate measure, because if the response to these stimuli is subject to habituation, infants who had a higher number of valid trials could not show a response (or they would show a diminished one) as a result of habituation. However, since neither level of movement, nor the number of trials significantly correlated with heart rate (all ps>.288), I did not include these measures in further analyses.

To test for an effect of the order of stimulation, I ran a 2x2x2 mixed ANOVA with touch type (slow and fast touch) and time (0-5s and 5-10s) as within subject variables, and order of presentation of the stimuli (slow or fast touch first) as between subject variable. This ANOVA showed no main effect of order ($F(1,53)$=.376, p=.543) and no significant interactions with order (touch type*order= $F(1,53)$=.001, p=.971; time*order= $F(1,53)$=.004, p=.951). Therefore, I dropped this variable from further analysis concluding that which touch infants received on the first trial did not affect the results.

6.2.5.2 Analysis plan

I performed a 2x2x2 mixed ANOVA with touch type (slow, fast) and segment of stimulation (0-5s, 5-10s) as within subject factors. Given infants in our sample ranged from 34 to 110 days of age we wanted to test for an effect of age; we therefore did a median split of our sample and obtained a younger (34-70.5 days) and an older group (70.5-110 days). Age, as a dichotomous variable, was entered as a between subject factor.

Next, to account for the two self-report measures, questionnaires' scores were added to the 2x2x2 ANOVA as covariates, in two separate models. To test for the influence of experienced touch, the PICS' total stroking scores were entered first in the model as a covariate. A separate model was then run with STQ scores to test for the influence of parental attitude to social touch on the heart. The model was run first with STQ scores from primary caregivers, then with STQ scores from their partners.

6.3 Results

6.3.1 Effect of age

A 2x2x2 mixed ANOVA was performed with touch type (slow and fast) and time (0-5s and 5-10s) as within subject variables, and age (younger, older) as between subject variable. This ANOVA showed a main effect of time $(F(1,53)=17.291, p<.001, \eta p^2=.282$; Figure 6.2), no main effect of touch type $(F(1,53)=.312, p=.579)$, and a trend towards an interaction between age and time $(F(1,53)=2.290, p=.090)$. The interaction reflects that responses in the first half of the stimulation are similar for younger (mean=.005) and older (mean=.005) infants while during the second half of the stimulation younger infants return to baseline levels (mean=-.001) and older infants show a more sustained response (mean=.003).
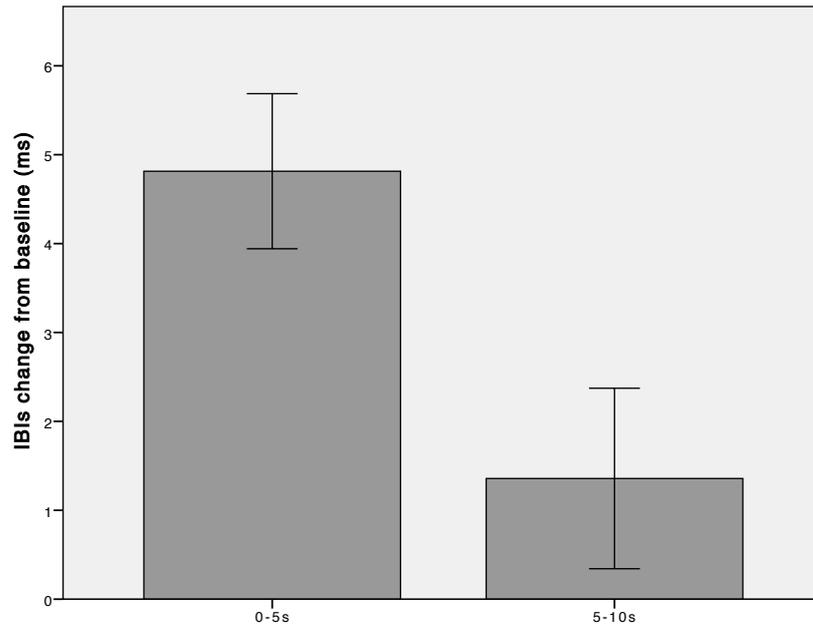
Figure 6.2 IBIs changes relative to baseline. Responses to slow and fast touch are averaged together. This measure was quantified subtracting the average of the IBIs over the last five seconds of baseline from each of the stimulation segments (0to5 and 5to10 seconds post stimulus onset). Positive values resulting indicate a heart rate decrease (IBIs are longer during the stimulation period compared to baseline). Error bars represent the standard error.

6.3.2 Effects of experienced amount of stroking

Scores within each of the four stroking items ranged between 2 and 5 (for details on each item see Table 6.2) The total stroking score had a normal distribution (see Figure 6.3) with mean=3.69 and SD=0.63. Total stroking scores were inserted as a covariate to the three-way mixed ANOVA. No main effect of stroking and no significant interactions were found (all ps > .4)

item	N	mean	SD
I stroke my baby's tummy	49	3.54	0.87
I stroke my baby's back	49	3.65	0.93
I stroke my baby's face	49	3.98	0.85
I stroke my baby's arms/legs	49	3.60	0.89
PICS' total stroking score	49	3.69	0.63
STQ - primary caregiver	54	25.22	9.91
STQ other parent	46	29.65	11.8

Table 6.2 Descriptive values the four items of the PICS relative to stroking behaviours, for the total stroking score, and for STQ scores from both parents.
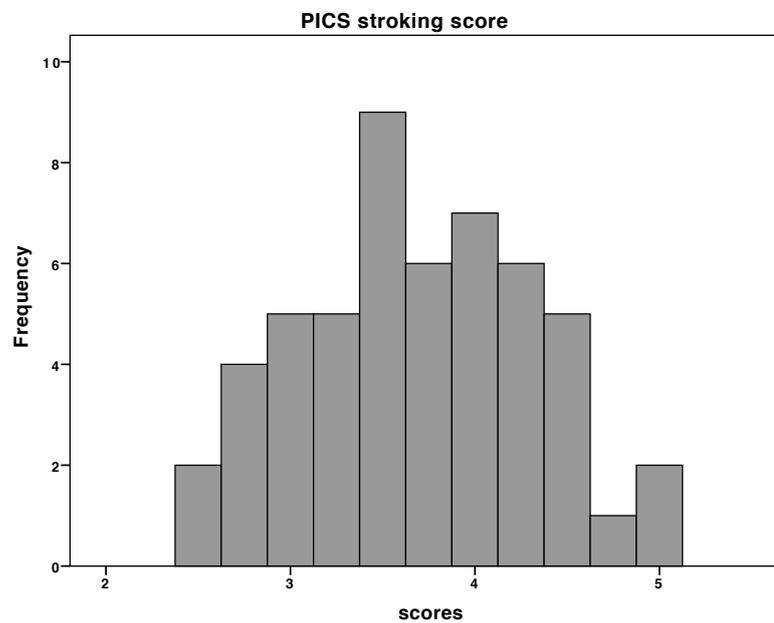
Figure 6.3 Frequency distribution of total stroking scores.

6.3.3 Effects of parental touch responsivity

The distribution of STQ scores from the primary caregiver (range=8-53, m=25.22, SD=9.91) had a positive skew, with more parents stating they enjoyed giving/receiving touch, while the distribution of STQ scores from the other parent (range= 7-55, m=29.65, SD= 11.8) being more evenly distributed (see Figure 6.4).

A two-sample t-test showed that there is a significant difference in STQ scores, with main caregivers being less aversive to social touch than their partners (t=2.325, p=.022, d=0.4). STQ scores of main caregivers and their partners are not correlated (p=.211).
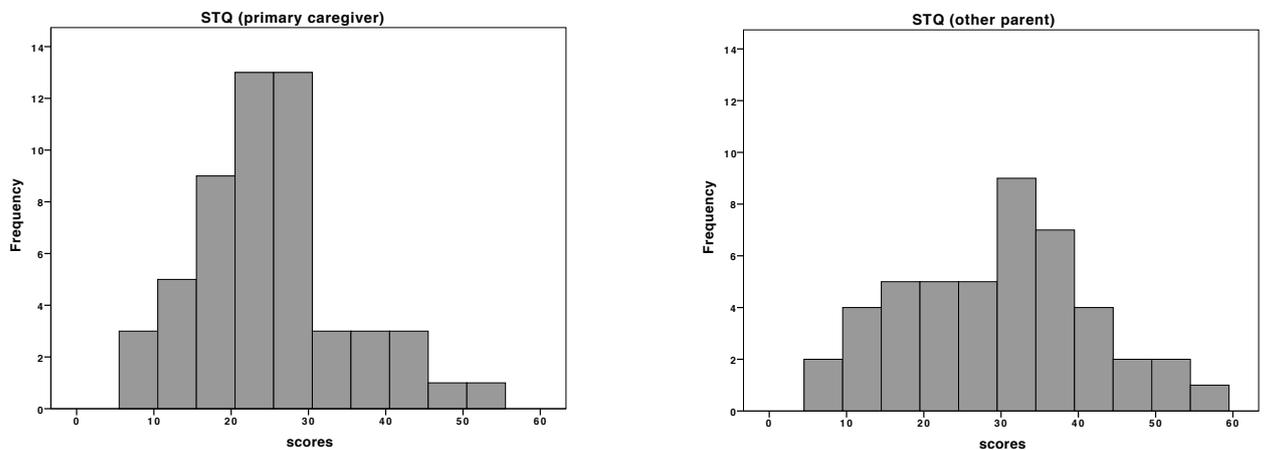
Figure 6.4 Frequency distribution of STQ scores from primary caregiver (left) and other parent (right).

6.3.3.1 Maternal STQ

I introduced first the primary caregiver's STQ scores as a covariate to the three-way mixed ANOVA. A significant interaction between touch type and STQ scores was found (F(1,50)=4.778, p=.034, ηp^2=.13), with STQ scores showing an association with slow but not with fast touch (see Figures 6.5 and 6.6). The scatterplots show that there is a negative, but non-significant, relationship between STQ scores and the response to slow touch (r=-.155, p=,262) (Figure 6.5-black line) but there is no relationship with fast touch (r=.11,

p=.425)(Figure 6.6-black line). This trend suggests that the better the parent's attitude to social touch, the larger the infant's heart rate decrease to affective touch (and vice versa, the more adverse the parent is to social touch, the larger the infants' cardiac acceleration to affective touch).

Further, a significant three-way interaction between touch type, age and STQ scores ($F(1,50)=8.463$, p=.005) was found. To untangle this interaction, I correlated STQ score to the response to slow touch in each age group separately. Looking at the two age groups separately I find that the negative relationship between STQ scores and the response to slow touch is driven by the younger group (r=-.539, p=.003; Figure 6.5-blue line) and absent in the older group (r=.008, p=.970; Figure 6.5-green line).

In addition to the relationship with the response to slow touch, I wanted to test whether maternal attitude to touch was also related to the infant's ability to discriminate between different touches. Thus, for each infant, I calculated an index of the ability to discriminate between slow and fast touch (subtracting their response to fast touch from their response to slow touch). Figure 6.7 shows the relationship between STQ scores and this index. Younger infants in the sample show larger heart rate decreases to slow than to fast touch if their mothers have lower STQ scores (r=-.603, p=.001) (otherwise if their mothers are more averse to social touch they are more sensitive to non-affective touch). In older infants this index is not related to the mum's STQ score (r=.104, p=.614).

6.3.3.2 Other parent STQ

I then introduced STQ scores from the other parent as a covariate to the three-way mixed ANOVA. The interaction effects found with scores from the primary caregiver were not replicated when the other parent's scores were instead used (touch type*STQ $F(1,42)=.596$, p=.445; touch type*age*STQ ($F(1,42)=.004$, p=.949). Since I could collect a lower number of questionnaires from partners compared to primary caregivers (46 vs. 54), I rerun the 2x2x2 ANOVA with STQ scores only from the primary caregivers whose partners had also filled the questionnaire in. I found that the original interaction effects remained significant (touch type *STQ $F(1,42)=4.212$, p=.046); touch type*age*STQ ($F(1,42)=7.786$, p=.008, ηp^2=.12).

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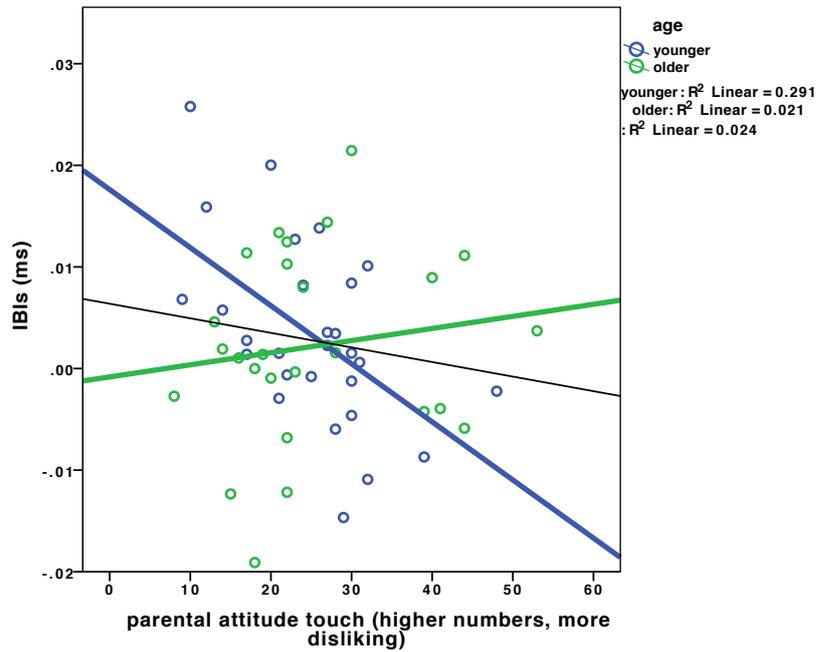
slow touch

Figure 6.5 Scatterplot depicting the association between primary caregiver's attitude to social touch (x axis) and infants' response to slow touch (y axis). On the Y axis is IBI changes relative to baseline in response to slow touch (averaged over the entire 10s trial sine no interaction with time resulted from the ANOVA). Blue dots represent younger infants and green dots represent older infants.

fast touch
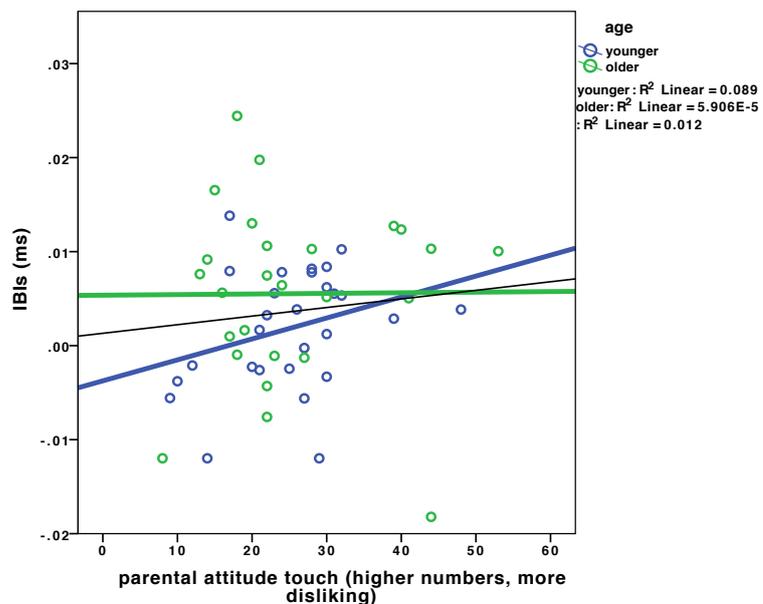
Figure 6.6 Scatterplot depicting the association between primary caregiver's attitude to social touch (x axis) and infants' response to fast touch (y axis). On the Y axis is IBI changes relative to

difference slow-fast
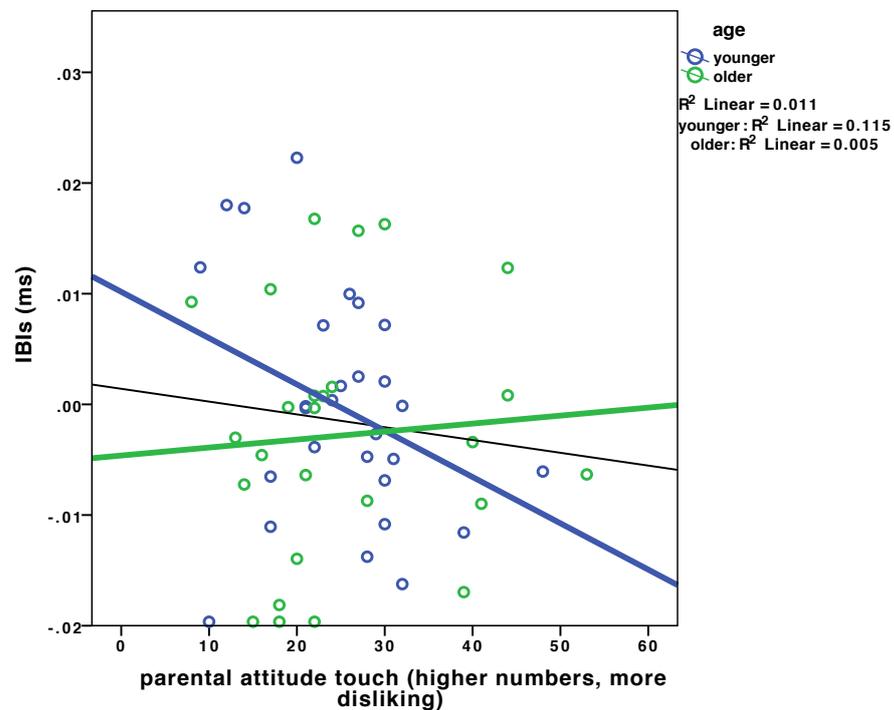
Figure 6.7 Scatterplot depicting the association between primary caregiver's attitude to social touch (x axis) and infants' discriminatory response to slow vs. fast touch (y axis). on the Y axis is the difference between slow and fast touch trials. Positive numbers indicate a bigger response to slow vs. fast touch and negative numbers indicate a bigger response to fast vs slow touch. Blue dots represent younger infants and green dots represent older infants.

6.4 Discussion

In the present study, I employed a physiological measure (heart rate) to assess infants' ability to discriminate affective from non-affective touch. I hypothesised that affective tactile contact would increase parasympathetic activity, decreasing heart rate, while the non-affective contact would have a lesser impact at the autonomic level. Contrary to this prediction, the results show that infants between 1 and 3 months process affective and non-affective touch similarly. Indeed, both touches have a transient effect on heart rate that does not remain sustained throughout stimulation.

This transient heart rate decrease observed here is a component of the orienting response (OR) (Graham & Clifton, 1966). According to Sokolov's theory (1963), the OR is the first response of the organism to a stimulus and it facilitates information processing by decreasing sensory thresholds. This is an early phase of information processing where the infant encodes some preliminary information about the stimulus and decides whether to allocate further mental resources to it (Kahneman, 1973). The OR in itself does not reflect an arousal decrease caused by a parasympathetic dominance; instead it was proposed that during orienting there is a transient increase of arousal (cortical areas may become activated, to indicate increased sensitivity to stimulation) accompanied by inhibition of the cardiac activity (Lacey, 1959); the phenomenon whereby different autonomic responses do not show a positive covariance takes the name of *directional fractionation* (Lacey, 1959). Therefore, this early response suggests that infants in our sample are detecting the tactile stimuli and evaluating both as non-harmful for the organism (an aversive stimulus elicits a defensive response indexed by an increase in heart rate) but they are not discriminating one from the other. In contrast with this result, significantly different orienting responses have been reported to CT-optimal and -suboptimal touches in adults (with larger heart rate decreases observed for the former; Pawling et al., 2017a, 2017b). Given that in infancy the orienting response is modulated by variables such as stimulus novelty, complexity and significance (for a review see Graham and Clifton 1970), it was reasonable to expect discrimination at this level. The lack thereof suggests that infants orient similarly to decontextualized affective and non-affective touch and, at least at the cardiac level of this early information processing stage, they are not recognizing the relevance carried by the CT-targeted touch.

Following a similar orienting response to both touches, I show that the initial deceleration is not sustained for either stimulation. In the introduction of this chapter I outlined two different mechanisms that could underlie a sustained response. If a sustained cardiac deceleration reflected a 'calming response', affective touch might have not triggered it because the current internal state of the infant did not 'require' a parasympathetic activation. While Esposito et al. (2013) observe a cardiac deceleration even in those infants who were calm before being carried, suggesting that the calming response is

independent of the initial state, we have to bear in mind that our affective touch stimulus differs from maternal carrying as it lacks the proprioceptive component. Indeed, the vestibular-proprioceptive input might be necessary for the calming response given that holding alone (somatosensory component only) did not afford the same effect (Esposito et al., 2013). However in Esposito et al. there is not a no-touch baseline, so probably holding induced a decrease compared to no-touch but this was not measured.

Alternatively, it could be that presenting the stimulus in isolation from other contextual cues hindered the arousal decrease. For the infant, tactile stimulation in absence of concurrent information about the agent of the touch might not be sufficient to elicit a calming response and top-down influence to evaluate who is eliciting such response might be necessary. Therefore, affective touch might elicit a parasympathetic activation when the infant needs soothing or, if in a neutral state, the multimodal component could be important.

On the other hand, if a sustained cardiac deceleration reflected sustained attention, I might not have observed it because the physical properties of our stimulus do not require that sustained attention is paid. Indeed, due to the rhythmic nature of the stroking, the information it conveys could be encoded after only a few repetitions. Studies that investigated sustained changes in heart rate indexing sustained attention employed stimuli with a larger amount of information to be processed (i.e. a segment of the Sesame Street cartoon, (e.g. Richards and Casey, 1992), or a toy to be manipulated (Lansink and Richards, 1997)). Therefore, in the context of attention, rhythmic stroking might not be a complex enough stimulus to pay sustained attention to and a complete encoding could have taken place during the orienting phase of information processing. In a similar fashion as above, it could also be argued that the infant needs further information besides the somatosensory input to evaluate whether the stimulus is worthy of sustained attention. Infants might be often exposed to CT-optimal touches (like the shuffling of soft, warm clothes on the skin), but pay attention to it only when the agent is a caregiver. In Fairhurst et al. (2014) 9-month-old infants could see both the stimulation and who was performing it, and the fact that they looked more at the brush during the CT-optimal touch suggests a role of sustained attention in eliciting a cardiac deceleration.

Despite the lack of differentiation between slow and fast touch in the whole group, individual differences were observed. In particular, there was a relationship between the primary caregiver's attitude to social touch and infants' response to slow touch. This association was present only for the younger infants in our sample (34 to 70.5 days). This is partially in contrast with results from Fairhurst and colleagues, which show this association in 9-month-old infants. Interestingly I did not find any relationship between infants' response to slow touch amount of affective touch experienced. It is possible that sensitivity to social touch is heritable and the genetic component prevails on the experiential one early on (the response is observed independently of the context), while contextual cues may play an increasingly important role in determining this response with age. However, I expand on findings from Fairhurst and colleagues, showing that this relationship is not replicated when STQ scores for the secondary caregivers were instead entered in the model. This result weakens the heritability hypothesis, (unless 'sensitivity to affective touch' is only inherited from the mother) and suggests a different mechanism. Infants spend the first months of life mostly with the primary caregiver (mum) and the amount of touch they are exposed to could be a function of the caregivers' attitude to touch. Thus, if a genetic component is not involved it could be that mothers with a better attitude to social touch also stroke their infants more and these infants are more sensitive to the slow stroking. The disappearance of the relationship between two and three months and its reappearance at nine, could reflect the fact that the frequency of stroking is not constant throughout the first year of life. While stroking could be more frequent in caregiving interactions over the first two months of life, it might give way to another form of affectionate touch after this point, to then be preferred again when the infant is older. This is only a speculative interpretation given I have no data available for infants between four and nine months.

Despite the lack of an association between maternal stroking and response to slow touch, the conclusion that experience does not influence sensitivity to affective touch is not granted. Indeed, it is possible that the self-report measure (PICS) employed here is not the ideal tool to capture the frequency of maternal touch in daily life. Future research should devise new and more accurate ways to measure frequency of mother-infant touch.

6.4.1 Limitations

The measure collected in this study (heart rate) on its own does not allow us to clearly conclude whether touch is increasing or decreasing arousal. In order to interpret the observed response in a conclusive way we need another autonomic measure such as skin conductance response (SCR) or pupil dilation. The only study that in adults concurrently measured heart rate and SCR in response to CT- and non-CT-targeted touch found than the former yields a larger cardiac deceleration and a smaller SCR than the latter (Pawling et al., 2017b) suggesting that both touches are arousing but affective touch is less so. The SCR finding was replicated shortly after by another group (Etzi, Carta, & Gallace, 2018). I cannot exclude that a different autonomic measure than heart rate could be better at revealing a differential response.

A further limitation to this study is that I did not record parent-child interactions with the dyads that took part in the study. Measuring the amount of stroking in these interactions could have elucidated whether stroking behaviours decrease in the older group. Furthermore, correlating amount of stroking to a) the mum's STQ score and b) the infant's response could have clarified the role of experience on the infants sensitivity to slow touch.

6.4.2 Summary

Based on the current findings I cannot support the hypothesis that affective touch decreases heart rate in infants. Possible explanations for this finding have been presented and new interesting research avenues can stem from this work. Exploring further the relationship between parental attitude to social touch and infants' sensitivity to slow stroking, manipulating age and contextual cues, could help elucidate the relative contributions of experience and heritability. Like previous chapters, this one also raises the question of whether stripping the touch of its contextual cues might hinder instead of unveiling its unique impact on the developing organism.

Chapter 7

General Discussion

The primacy of the sense of touch in development is an indisputable fact. Touch is the first sense to develop prenatally, thus the first sensations experienced in life are tactile. Then, once an infant is born nearly all episodes of parental care are mediated by (social) touch. It is therefore surprising that developmental psychologists have largely neglected touch in favour of vision and audition when studying perceptual development. As detailed in Chapter 1, animal models clearly showed the impact that social touch during early development has on a number of outcomes (behavioural and physiological). Striking effects of social touch, in line with animal models, have also been reported in human infants born preterm. In contrast to the study of newborns the role of social touch was far less investigated in typically developing infants. Behavioural research has been employed to describe the different roles social touch subtends in parent-infant interactions, however work that looked at how social touch impacts different physiological levels to try and pinpoint potential mechanisms for its effects is scarce. Given the currently limited understanding of the mechanisms that mediate the effects of social touch in typically developing infants (as highlighted by the wealth of behavioural studies reviewed in Chapter 1), I aimed to investigate how infants process one type of social touch (CT-afferent mediated affective touch) by measuring brain and autonomic responses.

The overarching aim of this thesis was to investigate how human infants process CT-afferent mediated affective touch. In the next section the experimental findings of this thesis are discussed in relation to the following questions (as outlined in Chapter 1):

1.   Do young infants exhibit cortical specialization to affective touch (human stroking) in the posterior superior temporal sulcus and inferior frontal gyrus? (Ch.3,4)

   1.1.   Is a difference in temperature sufficient to elicit differential responses in these regions? (Ch.3, Experiment 1)

   1.2.   Can differentiating affective and non-affective touch on more than one dimension facilitate discrimination? (Ch.4, Experiment 2)

1.3.   Do young infants display differential cortical responses to the stimulus contrast typically employed in work with adults: slow vs. fast brush stroking? (Ch.4, Experiment 3)

1.3.1   Do infants process CT-optimal touch delivered through a brush in the same way as stroking performed with a human hand?

1.4.   How does the processing of affective touch develop across the first year of life? (Ch.4, Experiment 4)

2.   Do infants display differential cardiac responses to affective and non-affective touch? (Ch.5,6)

1.1.   Does affective touch promote focused attention, via a decrease in arousal (indexed by heart rate) in infants between 6 and 10 months? (Ch.5, Experiment 5)

1.2.   Are younger infants more likely to display differential cardiac responses to affective touch (Ch.6, Experiment 6)?

7.1 Affective touch processing in the developing brain

As discussed in Chapter1, neuroimaging studies of affective touch processing in adults have shown the involvement of a widespread network of areas beyond the somatosensory cortex (see Chapter 1, section 1.4.2). Key nodes of the social brain have consistently emerged from these works, including the pSTS and the IFG (McGlone et al., 2012; Gordon et al., 2013; Voos et al., 2013). One study indicated that school aged children recruit a network of regions (including pSTS) similar to adults for the processing of affective touch with frontal responses not being consistently observed until adulthood (Bjornsdotter et al., 2014). However, to date, only a handful of works aimed to unveil the cortical underpinnings of affective touch in infancy, using a variety of stimuli and experimental settings (Tuulari et al., 2017, Jönsson et al., 2017, Miguel et al., 2017, Kida and Shinohara, 2013, Saito et al., 2009). The majority of these studies used fNIRS and recorded responses from different cortical regions or hemispheres (see Table 7.1). Taken together these studies led to inconsistent

findings and did not provide coherent answers relative to how affective touch is processed in young infants.

The first part of this thesis (Chapters 3 and 4) aimed *1)* to characterize the brain processing of affective touch in young infants (5-7 months old) and *2)* to investigate how these responses change across the first year of life (testing 10 months old infants). fNIRS was employed to measure cortical responses to three stimuli contrasts, where affective touch (defined as touch optimal for eliciting activation of CT-afferents) was contrasted to non-affective touch.

7.1.1 Processing of affective touch in 5-7 months old infants. Is context important?

The findings revealed that adult like responses are not consistently observed in infants aged 5 to 7 months over the cortical areas investigated in these experiments. While a response to affective touch was observed in Experiment 1 both over IFG and pSTS/TPJ, the same regions also showed activation to the non-affective touch. This similar pattern of responses is open to two opposite interpretations: 1) responses over these areas, at this age, are generic for affective and non-affective touch and indicate that specialization has not yet occurred (these areas are sensitive to touch but not yet selective to affective touch), or 2) specialization has already occurred and the observed responses actually reflect affective touch processing. Indeed, I advanced the possibility that in the absence of visual access to the stimulation, both stimuli (hand and spoon) have been processed as affective touch. To clarify whether these cortical regions display differential responses to affective vs. non-affective touch, two other stimuli contrasts were explored in Experiments 2 and 3. However, rather unexpectedly, the new findings did not help answer the questions that had arisen from Experiment 1. First of all, the clear responses elicited by both stimuli in Experiment 1 were not observed for affective touch in Experiments 2 and 3. In Experiment 2 responses to affective touch were absent and in Experiment 3 they were limited to one channel. Second, non-affective touch elicited widespread responses in both experiments. Thus, from these two experiments it emerges that at this age the processing of affective touch can undergo interference from other non-affective touch stimuli.

In summary, the regions that that I hypothesized would show selective responses to affective touch responded to:

- *Affective touch in experiment 1 (pSTS/TPJ, IFG)*
- *Non-affective touch in experiments 1, 2 (pSTS/TPJ, IFG) and 3 (pSTS/TPJ)*

In light of these findings it would seem that at this age while adult-like affective touch processing can be observed in certain circumstances, cortical specialization has not fully developed yet.

Given the inconsistency that emerged from the handful of published studies to date (see table 7.1), I set out to clarify how affective touch is processed in the developing brain through the set of experiments in this thesis. However, instead of adding clarity, this work contributed to current knowledge with findings both unexpected and not easy to interpret. CT-optimal touch was shown to activate the insula in newborns (Tuulari et al., 2017) and the insula and the posterior temporal cortex in 2-months-old infants (Jönsson et al., 2017). These two pieces of evidence suggest that selective responses in the temporal cortex may emerge during the first two months of postnatal life. However, activation of the temporal cortex is no longer observed in 7 months old infants (for neither affective nor non-affective touch; Miguel et al., 2017). With my studies, I show that at 5 months the posterior temporal cortex responds to CT-optimal touch (Experiment 1) *but also* to CT-suboptimal touch (Experiment 2). The latter finding was replicated with the same stimuli used in Jönsson et al.'s work with 7 months old infants (Experiment 3) corroborating the idea that while specialization is underway paradoxical responses are observed.

Could (the absence of) contextual cues explain the lack of response to affective touch in Experiments 2 and 3? Should this be the case, what contextual cues do infants need in order to process affective touch? Is the identity of the person important (i.e. caregiver vs. anyone else) or is it sufficient to see who is performing the touch independently of their level of familiarity with the infant? For example, STS responds to social information in the visual and auditory domain even if it is not the caregiver's face or voice that the infant is exposed to (e.g. Farroni et al., 2013; Lloyd-Fox et al., 2009, 2011; Blasi et al., 2011). It is possible that both familiar (the caregivers) and unfamiliar people can represent a source of visual and auditory social cues for young infants. In

contrast, the main source of affective touch for infants could be confined to their caregivers and perhaps young infants have learnt to associate affective touch to the presence of their caregivers.[32] Therefore, differently from vision and audition, they might only process the touch as affective when this is performed by the caregiver. According to this idea, in Jönsson et al.'s study (2017), infants might have displayed cortical selectivity to affective touch because they perhaps believed that the touch was originating from the mother, given that they were being held in their mothers' arms. Had I provided the infant with visual access to the caregiver, would it have been sufficient to reverse the pattern of responses observed in Experiments 2 and 3? I showed that in the absence of contextual cues, the most intense stimulation elicits the largest activation. *Could top-down mechanisms prevent the bottom up processing of these responses?*

However, in Experiment 1 responses to affective touch were observed despite the experimental setting being also stripped of social cues. Thus, contextual cues might facilitate the infants processing of affective touch if there is a 'competition' between affective and a *novel* or *more intense* form of touch. Therefore, if the present experiments were rerun changing the experimental setting to lead the infants to believe they are touched from their parents (still not being able to view the stimuli) I would expect the results to change as follows. For *Experiment 1*, since tuning to the temperature has not developed yet, I would expect to observe the same results. For *Experiment 2*, I expect to see a response to hand stroking in both IFG and pSTS/TPJ. However, I would not necessarily expect to no longer observe activation to the electric toothbrush. Indeed the 'extreme' novelty of this type of stimulation could still lead to widespread responses. Especially if the responses observed are originating from adjacent somatosensory cortices (as discussed in Chapter 4) I would still expect to observe these despite context-related changes. For *Experiment 3*, I

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[32] Infants might interact with people other from their caregivers (and thus see them smiling or hear their infant-directed speech) while being held by the caregiver. Or they could be exposed to visual and auditory cues on 2D screens (cartoons or video calls). So, while they might be touched by few people that they learn to associate the touch with, the number of people they see/hear is larger and thus not such an as strict association is formed.

would expect to see the same pattern observed in Jönsson et al.'s work, with selectivity responses to affective touch.

7.1.2 Developmental effects on the processing of affective touch

Findings from Experiment 4 seem to suggest that the widespread response to non-affective touch observed in Experiment 3 has subsided by 10 months of age. Indeed 10-months old infants show no-response to non-affective touch and activation to affective touch in the pSTS region similar in magnitude to the one observed in 7-months-old infants. Therefore, while the response to affective touch has not increased (in terms of number of active channels) the unexpected larger response to non-affective touch is no longer present. Could this finding be evidence of the ongoing tuning to affective touch in pSTS?

As part of a longitudinal study, Miguel's group tested the same infants at 7 (Miguel et al., 2017) and 12 months. Although the paper with findings from the older age group is not published yet[33], the first author shared these findings with me: compared to 7-months-olds (that did not show pSTS activation to affective touch), 12-months-olds display large responses to affective touch over the pSTS region. This result lends support to the idea that across the second semester of postnatal life the (cortical) processing of touch undergoes development. While at 10 months (vs. 7) I do not yet observe an increase in the response to affective touch, perhaps an indication of ongoing developmental change resides in the absence of the response to non-affective touch.

An obvious next step would be to test the slow vs. fast touch contrast with 12-month old infants. From such an experiment, I would expect (for the posterior temporal region) to *1)* replicate the finding from Experiment 4 relative to non-affective touch and *2)* observe an increase in response to affective touch.

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[33] As per personal communication with the first author, the paper was accepted in *Social Neuroscience* and is currently undergoing the editing process.

study	Age	Technique used	Brain regions measured	Social touch	Non-social touch	location stimulated on the body	Areas with social touch selectivity	Areas with non-social touch selectivity
Saito et al., 2009	2-11 days	fNIRS	anterior prefrontal cortex	3cm/s stroking with cotton	3cm/s stroking with soft plastic	left ventral forearm/ left mid- cheek (between participants)	aPFC: Cotton>wood on forearm; plastic>wood on cheeck	/
Tuulari et al., 2017	11-36 days	fMRI	ROIs selected: postcentral gyrus/ insular cortex	3cm/s brush stroking	/	right anterior shin	postcentral gyrus/ insular cortex	/
Jonnson et al., 2017	2 months	DOT	*left* temporal cortex	2cm/s **brush stroking**	20 cm/s **brush stroking**	right forearm	left middle temporal gyrus extending into STS / left insular cortex	/
Kida and Shinohara, 2013	3,6,10 months	fNIRS	anterior prefrontal cortex	gentle stroking with velvet	gentle stroking with wood	left hand palm	aPFC only at 10 months	/
Experiment 1	5 months	fNIRS	*right and left* inferior temporal cortex from IFG to pSTS	5cm/s hand stroking	5cm/s spoon stroking	Right or Left upper arm	IFG and pSTS/TPJ?	/
Experiment 2	5 months	fNIRS	*right and left* inferior temporal cortex from IFG to pSTS	5cm/s hand stroking	30cm/s electric toothbrush stroking	Right/left upper arm	/	IFG and pSTS/TPJ
Miguel et al., 2017	7 months	fNIRS	*right* temporal cortex/ left postcentral gyrus	8cm/s **brush stroking**	tapping with a squared-shape piece of wood	right forearm	/	/
Experiment 3	7 months	fNIRS	*right and left* inferior temporal cortex from IFG to pSTS + somatosensory	3cm/s **brush stroking**	30 cm/s brush stroking	right forearm	/	pSTS/TPJ
Experiment 4	10 months	fNIRS	*right and left* inferior temporal cortex from IFG to pSTS + somatosensory	3cm/s **brush stroking**	30 cm/s brush stroking	right forearm	pSTS/TPJ?	/
Miguel et al., 2018	12 months	fNIRS	*right* temporal cortex/ left postcentral gyrus	8cm/s **brush stroking**	tapping with a squared-shape piece of wood	right forearm	pSTS	/

Table 7.1 Table with neuroimaging studies that investigated affective touch processing in infancy. Greyed areas represent Experiments from this thesis and the relative findings.

7.2 Autonomic processing of affective touch in infancy

Across studies of animals and human adults, social touch has been shown to increase parasympathetic activity, measured via decreased blood pressure and heart rate. In particular, in adults, heart rate decreases have been reported to both prolonged (15s; Triscoli et al., 207) and brief (3s; Pawling et al., 2017a) presentations of CT-afferent mediated affective touch. Does social touch have the same effect also in young infants? Only one study to date has measured heart rate changes to CT optimal touch in infants and, in line with findings in adults, also showed heart rate decreases to 10s long stimulation (Fairhurst et al., 2014). With work from the present thesis I set to collect further evidence on how CT-afferent mediated affective touch impacts the ANS in infancy.

Thus, Chapters 4 and 5 aimed *1)* to investigate heart rate changes to affective touch in three age groups (1-3, 6-7 and 8-10 months old) and *2)* to further investigate whether changes in arousal (indexed by heart rate) map onto changes in visual attention. The most important finding that emerged from this work is that none of the age groups displayed differential responses to affective vs. non-affective touch, in terms of heart rate. In addition, sustained heart rate decreases were not observed to either type of touch. Thus, the experiments with three different age groups and employing two different experimental paradigms (one between-subjects with concurrent visual attention task, and one within-subjects with no attention task) led to results that stand in contrast with the only other piece of evidence to date (Fairhurst et al., 2014). Certainly, these findings invite us to question the idea that affective touch increases parasympathetic activity in infancy as it does in adulthood (and in animals). Fairhurst et al. (2014) suggested that infants are sensitive to affective touch, however the differential response (and the deceleration to affective touch) they observe is no longer present when some of the experimental settings are changed (as in Experiment 5 and 6). This suggests that the effects of affective touch are not measurable regardless of the context in which the touch occurs but are instead mediated by other factors. The presence of affective touch is not sufficient per se, as animal models suggested

was the case (as discussed in Chapter 1, section 1.1..2), but top down mechanisms might be necessary to perform correct interpretation of the touch and display the appropriate response.

The current findings warrant further work to understand the role of different contextual factors in modulating the cardiac response to affective touch. In the Fairhurst et al. study (2014) infants were sitting on a Bumbo chair placed on the mother's knees and mothers were holding the infants' leg to prevent movement and artifacts in the pulse oximeter signal. Is *awareness of close maternal presence* necessary to observe the predicted effects of affective touch? Even though is a different type of measure, this factor was not sufficient in itself to elicit social brain responses in Experiments 2 and 3 (where infants were sitting on the mother's lap). Would the same results be replicated if the infants were instead sitting by themselves at a distance from the mother? The other difference compared to the experimental settings used here is that in Fairhurst's study, infants could see the experimenter and the stimulation. Thus, having *contextual cues about the touch* facilitates its processing. In particular, in the Fairhurst et al. infants looked longer at affective compared to non-affective touch. This piece of evidence, however, raises questions regarding the nature of the cardiac deceleration: is this an index of an increase in parasympathetic activity or of active information processing (sustained attention)? Does the touch elicit a soothing function (indexed by parasympathetic activity) or does the infant allocate cognitive resources to the touch, thus entering a sustained attention phase? If the deceleration is the result of the former mechanism, it would imply an active (bottom-up) role of this form of touch on the organism. Instead, if an attention mechanism is involved, it means that the infant has decided to allocate attention to the touch. The observed heart rate deceleration on its own does not warrant support of either hypothesis. If this deceleration was still observed in a setting where the infant could see the experimenter, but not the touch, then it would support the soothing function of social touch. On the other hand, to test whether active information processing can explain the deceleration, resistance to distraction during touch stimulation could be measured (which would represent a modification of the 'interrupted stimulus' paradigm; e.g. Richards, 1985). Longer latencies to disengage from the touch and orient to the distractor would index sustained attention.

Besides the finding of a lack of discrimination between affective and non-affective touch, which is common to both Experiment 5 and 6, other findings resulted from each experiment. In Experiment 5 I showed that the time course of the heart rate response is different in touch compared to no-touch conditions. The results suggest that the presence of touch during the visual orienting task dampens the cardiac response elicited by the task on its own. Furthermore, reaction times in the visual attention task were not modulated by either presence/absence of touch or touch type. Thus, the differences in heart rate to touch and no touch blocks did not map into differences in reaction times. In Experiment 6, again, I found a similar orienting response to both touch types. Further I found that individual differences in the response to affective touch are associated with maternal attitude to social touch. The more positive the maternal attitude to touch, the larger the infants' heart rate decrease to affective touch. This finding needs to be further explored to determine whether sensitivity to touch is inherited or mediated by a more frequent exposure to touch (do mothers with a better attitude to social touch also spend more time in contact with their infants?).

7.3 Theoretical implications and questions for future research

The four experimental chapters of this thesis attempted to provide answers to the research questions raised in Chapter 1. The findings from each experiment suggested novel questions. The present section will address the theoretical implications that emerged from this research and indicate possible directions for future research on affective touch in infancy.

7.3.1 Insula (and other brain regions) involvement in infancy?

In the present research project fNIRS was employed to probe cortical responses to touch. My research showed that, in the age groups tested, specialization in inferior frontal and posterior temporal cortices is still underway. However, investigation of the cortical underpinnings of affective touch was limited to

these regions and how touch is processed at other nodes of the network identified in work with adults (described in Chapter 1) is a question that still remains unanswered.

First, it would be important to measure responses to touch in **posterior insula**, the first cortical target of the CT-system (e.g. Olausson et al., 2002, 2010) during the first year of life. While I did not observe selective responses to affective touch in inferior frontal and posterior temporal regions it is possible that discrimination took place at the level of the insula in Experiments 1, 2 and 3. It was suggested that CT-optimal touch activated this area in newborns (Tuulari et al., 2017) and in 2-month-old infants (Jönsson et al., 2017). Being able to replicate activation in this region to CT-optimal vs. suboptimal touch across early development would provide stronger evidence that affective touch processing depends on the CT-system. Indeed, the use of microneurography to assess the existence and functionality of CT-afferents in infants is not a viable option. Further, it is possible that specialization in this region occurs earlier in development compared to the associative higher-order brain regions investigated here. While probing responses to touch in the insula certainly is an exciting research avenue to pursue, methodological limitations are to be considered. Indeed fNIRS' spatial resolution only allows measurement of the surface layers of the cortex and the insular cortex is too deep in the brain to be probed with this technique in infants older than approximately 2 months of age (due to changes in the optical properties of tissue and size of tissue layers). Although fMRI would represent the obvious candidate, providing whole brain measurements, a number of challenges to data collection have to be kept in mind. Functional fMRI studies have been successfully run with infants using auditory stimuli (e.g. Dehaene-Lambertz et al., 2006; Blasi et al., 2011), but the use of active tactile stimulation could increase the chances of waking the infant during the study. Further, the use of more than one stimulus (an affective and a non-affective touch) using approximately 30s long blocks (stimulation + baseline) would increase the length of the experiment increasing the chances of awakening the infant, and therefore reducing the chances of collecting a sufficient number of valid trials per condition. Possibly due to this reason, Tuulari et al. (2017) only used CT-optimal touch, thus from this study it cannot be concluded whether at birth the insula displays differential responses to

affective and non-affective touch. Therefore, while it might prove to be a long process, studies with fMRI should be pursued to better understand selectivity to affective touch in the insula during development (and in other areas as well that cannot be measured by near infrared light, e.g. the anterior cingulate cortex).

Future research using fNIRS should aim at measuring from a larger number of brain areas other than those measured here. Measuring other cortical nodes of the network that supports affective touch processing in adults would help to understand whether specialization has occurred outside of IFG/pSTS. For example, one region to probe would be the orbitofrontal cortex (OFC) that in adults was shown to respond to affective touch (Francis et al., 1999) and to a broad range of rewarding stimuli (Rolls et al., 2004). In infants, selective responses in this area were reported to pleasant touch (although CT-suboptimal) at 10-months (Kida and Shionhara, 2013) and to visual stimuli depicting smiling mothers at 11-months (Minagawa-Kawai et al., 2009). This area is described as part of the "social brain" network (Adolphs, 2003) and it was suggested to support the formation of mother-infant attachment (Nitschke et al. 2004, Minagawa-Kawai et al., 2009). Thus, it would be compelling to further investigate its involvement in the processing of social touch and its potential selectivity to maternal touch. However, to do so headgear designs need to be optimised in order to be able to increase the number of optodes while maintaining optimal adherence to the scalp and without adding too much weight on the subject's head (therefore developing lightweight fibers or wearable devices). Ideally responses would be probed from the entire cortex, but since this is currently unfeasible, precedence should be given to certain areas.

In addition to measuring the insula and regions of the social brain, probing of **somatosensory cortices** (SI/SII) should be prioritised in all future work on touch using fNIRS. While this region would not discriminate affective from non-affective touch, recording its activity would further our understanding of the development of responses to touch in SI/SII, e.g. relative to somatopic organization. SI regions were targeted using fNIRS only in studies of preterm or newborn infants (e.g. Bartocci et al., 2006; Shibata et al., 2012). Accumulating data from this region across early development would complement the knowledge we currently have from other methods (EEG, MEG)

on touch processing. SI/SII were shown to respond to affective touch from childhood to adulthood, and there is no reason not to expect the same responses also in infancy. Unexpectedly these responses were not observed in Jönsson et al. (2017) and in Experiment 3 and 4 of the current thesis. Did this occur because responses took place in a region of the somatosensory cortex not probed by the optodes? Perhaps the forearm (body part stimulated in Jönsson et al., 2017) and the upper arm (stimulated in Experiment 3 and 4) are represented closer to the brain midline during early development. Future work should clarify this.

7.3.2 Affective touch and parasympathetic activity

In the present thesis heart rate was used to index the autonomic system and specifically heart rate decelerations were thought to reflect parasympathetic activity. However, it could be argued that a better way to probe parasympathetic activity is via measuring heart rate variability (HRV). Higher HRV is associated with higher parasympathetic activity. Due to the experimental design imposed when measuring HRV in response to a stimulus where HRV has to be measured at three time points (baseline-*resting HRV*, event-*reactivity HRV* and post event-*recovery HRV*) and each segment needs to be at least 1 minute long, it was not possible to extract this measure from the data collected for Experiment 5 and 6. Yet, these two measures should be related to one another with stimulus related cardiac decelerations being accompanied by an increase in HRV. This relationship was shown in a study of non-human primates, where during six minutes of grooming (performed by an experimenter) heart rate decreased and HRV increased (Grandi & Ishida, 2015). A similar paradigm, involving longer stimulation, could be pursued with human infants in order to clarify the involvement of the parasympathetic system in mediating the effects of touch. Within subjects designs comparing affective and non-affective touch would not be possible and between-subjects designs would probably be necessary.

HRV can be measured both in response to a stimulus and at baseline. Baseline HRV could be a useful measure to have to complement studies of heart rate. Individual differences in baseline HRV could explain individual differences

in heart rate changes to touch. For example, in the field of attention HRV was found to correlate with cardiac decelerations during sustained attention in infants between 8 and 26 weeks (for a review see Richards and Casey, 1992). Infants with high baseline HRV had larger and more sustained heart rate responses in sustained attention. Future work might perhaps discover that experiencing higher levels of affective touch in daily life (see next section) leads to increased baseline HRV which in turn could lead to higher sensitivity to affective touch.

7.3.4 Naturalistic observations of touch

One of the questions that inevitably arose during this research project is the one that pertains the frequency of affective touch in infancy. As I was faced with unexpected findings (of a lack of cortical or physiological prioritization of affective touch) I started questioning how often infants experience CT-optimal touch in their daily lives. Are infants not displaying the expected response to affective touch at the different observational levels because they are not as familiar with this type of touch as I had assumed? While the self-report questionnaire revealed that mothers frequently engage in stroking different parts of their infants' bodies it is possible that a one-off questionnaire is not the ideal tool to capture frequency of touch and its variability. Questionnaires and parent-child interactions recorded at the lab should be complemented with more frequent and fine-grained measures of daily touch. Researchers should develop apps that parents can use to record episodes of touch with their infants. Besides recording the type of touch used (affective, instrumental, playful) parents could add information relative to the infant (e.g. mood) or the situation in which the touch occurred. Large amounts of this type of data collected would provide us with a 'touch map' that would help us to better understand functions of touch (studying associations between touch type, infant characteristics at the time of touch and situation in which the touch occurred). If these data can be collected for several months of postnatal life then we would also further our understanding of how frequencies and functions of different touch types change across development.

I believe that all research projects on social touch in infancy should strive to measure the amount of contact an infant is exposed to with as much detail as possible. Indeed, in animal models natural variations of tactile interactions between the mother and the pup have shown to predict outcomes both at the behavioural and at the physiological level (e.g. Liu et al., 1997; Laudenslauger et al., 1993). The natural variation of contact early in life could be used to explain individual differences in responses to touch as well as individual differences in baseline HRV or in stress reactivity. For example, the results of Experiment 6 raised the question of whether sensitivity to touch is inherited or mediated by experience of touch. Measuring touch frequency in the way proposed here would have helped answer this question.

One study that represents a step in this direction used an electronic diary for four consecutive days to quantify *'any caregiving carrying or holding that involved body contact'* and related this measure to infants' distress and epigenetic measures (Moore et al., 2017). The authors found that differentially methylated regions were identified across the entire genome between high and low contact groups.

7.4 Concluding remarks

I opened this thesis asking two broad questions, one stimulus-related (*what defines social touch?*) and one that addresses the mechanisms specific to social touch (*how does social touch promote development?*). I attempted to answer the first question advancing the idea that infants might discriminate touches that signal the availability of a caregiving mother (and thus a safe environment in which to thrive) from more general stimulation. I thought that one specific form of affective touch (CT-afferent mediated) could be a good candidate to represent caregiving touch. The effects of CT optimal touch were thus measured at different observational levels (brain, autonomic system and visual reaction times) to understand through which mechanisms social touch promotes development (to answer the second question).

In light of the novel findings that resulted from this thesis I now question whether dynamic gentle stroking is a form of social touch that mediates positive effects on development. It is the growing body of inconsistent findings across

observational levels that leads me to reconsider the role of CT-optimal touch in early development. Indeed, while some studies found a selective response to this type of touch (Jönsson et al., 2017; Fairhurst et al., 2014) others did not (Miguel et al., 2017; Experiment 2, 3, 5 and 6 from this thesis). Overall, the response to affective touch across the first year of life proved elusive.

In comparison, at the brain level, social selectivity to visual stimuli in the temporal cortex was replicated using a wide range of stimuli with evidence from shortly after birth (e.g. Farroni et al., 2013; Grossman et al., 2008; Lloyd-Fox et al., 2009). One question that inevitably arises is why within the same cortical region –pSTS/TPJ- should (gradual) specialization to social visual stimuli emerge sooner than to social touch? Given that infants experience an equal (as in highly abundant) amount of social visual and tactile stimuli, why should the social brain start tuning to one type of social stimulation sooner than the other? Is social information channeled through vision more important than that channeled through touch? Are the social signals encoded in faces (such as eye-gaze) more beneficial for infants' development and thus require processing precedence over social signals coming from other sensory modalities? While I do not have answers to these questions I believe that social signals in all senses carry positive values of similar importance. For example, eye-gaze is crucial for its communicative functions. It is considered the most powerful mode of establishing a communicative link between humans (Kampe et al., 2003) and its function to direct attention to target objects was shown to facilitate object processing (e.g. Reid et al., 2004) and thus it can be important for aspects of development such as learning. However, touch is also a primary mode of communication, and as emerged from the bulk of behavioral studies reviewed in Chapter 1 it is used by the caregiver to regulate the infant, communicate affection and direct attention (e.g. Stack and Muir, 1992). If these two signals are both essential to development in ways that complement one another it is difficult to explain the delay in cortical specialization to social touch compared to visual social stimuli. Touch was actually shown to increase social attention (eye contact and attention to the face; Roggman and Woodson, 1989; Pelaez-Nogueras et al., 1996; Stack and Muir, 1992). This suggests that often times when infants process social touch, they at the same time also process social signals through vision (caregiver's face and social signals within it such as eye

gaze) and through audition (caregiver's voice). Therefore, the lack of cortical specialization to CT-optimal touch possibly until the end of the first year of life, (against early emergence of specialization to social visual signals) together with the lack of selective responses to CT-touch at the autonomic level brings me to reconsider the choice of this stimulus. While I am not discarding the importance of affective touch in the form of gentle stroking, it is perhaps another type of social touch that positively impacts early development and that young infants are most sensitive to. While active stimulation is crucial to rodents' development, in humans this specificity might have been lost in favour of a less specific form of social touch. Touch is for young rodents, who are born blind and deaf, a channel of communication of paramount importance. In contrast, as soon as human infants are born they can experience the world through all sensory modalities. Therefore, the effects that in rodents are specific to active tactile stimulation in humans might be obtained via concurrent input to multiple sensory channels (tactile, visual, auditory). Physical and physiological wellbeing in human infants could be promoted by the frequent presence of a caregiver which can be experienced, for example, as the tactile sensation of being held, accompanied by the sight of the caregiver and the hearing of their voice. Thus, as concerns touch, it is possible that physical contact which signals the presence of the mother is sufficient to promote development. After this long journey we might come to discover that Harry Harlow, whose experiments opened this thesis, had the answer to the question *what defines social touch?* Contact comfort. One possibility is that the organism tunes to contact first while selective responses to other forms of social touch take longer to develop, are context dependent and modulated by top-down cognitive mechanisms.

The possibility that body contact is the form of social touch most important for development is difficult to test via measuring specific physiological responses to it (vs. another form of touch). Instead, future work should try and capture the amount of contact an infant is exposed to. Variability in this measure could be used to predict a multitude of outcomes such as baseline HRV, stress reactivity and even degree of selectivity so social stimuli on other modalities.
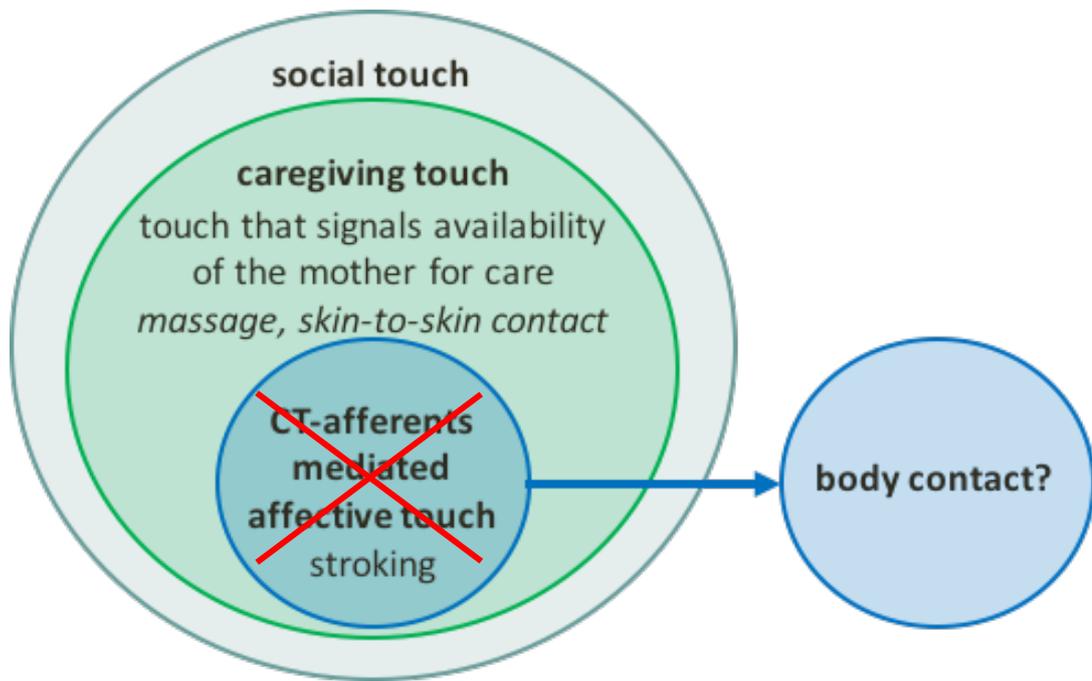
Figure 7.1 Diagram depicting subsets of social touch, with updated hypothesis regarding form of touch that promotes development

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