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Heart Rate Response in Newborns to Relatively Strong and Mild Trigeminal Odorants

Změny v Srdeční Frekvenci Novorozenců v Reakci na Odoranty s Relativně Silnou a Slabou Trigeminální Komponentou

Diplomová práce

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ABSTRACT

The widely accepted view nowadays is that experiencing odours as rather pleasant or unpleasant is, to a certain degree, shaped on a daily basis through individual experience within one's culture via evaluative conditioning or, rather marginally so, via mere exposure to that certain odour. In other words, humans are not born with any fixed set of olfactory likes or dislikes but rather, they acquire them throughout their lifetime. However, olfactory sensation is not a "pure" percept, as odorant stimuli generally elicit a qualitative percept of an odorant — generated mainly by the olfactory nerve — as well as some degree of chemesthesis — a tactile confound of the odour generated mainly by the trigeminal nerve. The olfactory and trigeminal system exhibit complex interactions at both the peripheral and central level of chemosensory processing, which is also reflected in perceptual characteristics of the final percept, including perceived pleasantness (hedonics).

If the olfactory contribution alone does not easily predict neonatal odour hedonics, due to newborns' limited previous exposure to chemosensory inputs, one may hypothesize that together with the strength of the trigeminal contribution they may form a significant factor affecting neonatal appetitive/aversive responses to odours. In the present study, odour hedonics has been operationalized in terms of autonomic responsiveness, namely heart rate variation. In adult studies, heart rate has been shown to correlate with perceived pleasantness, with unpleasant odours eliciting greater heart rate acceleration. In particular, one may expect that an intense, relative to a mild, trigeminal input will trigger a stronger defensive response (heart rate acceleration) in newborns, with no previous experience needed. To test this, we explored whether unfamiliar odours with contrasted trigeminal intensity (strong vs. mild) differentially elicit heart rate variations indicative of arousal magnitude.

Fifty 2- to 3-day-old newborns (26 F) were presented birhinally with three stimuli each — 1 relatively strong and 1 relatively mild trigeminal odorant in randomized order together with one blank stimulus. Thus, each presentation entailed three consecutive trials. For each trial a general linear model was run with continuous heart rate measurement averaged into 8 repeated measures over 10 seconds each as within-subject variables, odorant (mild/strong trigeminal stimulant/blank stimulus) and newborn sex as between-subject factors, and the

averaged 80 s baseline heart rate as a covariate. The analyses revealed a significant interaction of the odorant with the course of heart rate variation in the first trial. Further, repeated planned contrasts showed a significant difference between odorants across repeated measures circa half a minute after presentation. Such significant results were not found in the consequent trials.

The findings of this study might suggest an asymmetric processing of odours in the newborns at the heart rate level depending on the contrasting trigeminal component of the odorants. This asymmetric processing of odours might play a role in the formation of olfactory hedonics. However, our findings do not support the hypothesis that odours stimulating the trigeminal nerve more will elicit heart rate acceleration indicative of arousal magnitude.

Key words:

Chemoreception, Chemesthesis, Olfaction, Olfactory nerve, Trigeminal nerve, Heart rate response, Newborns, Hedonics

ABSTRAKT

V dnešní době se předpokládá, že vnímání pachů jako spíše příjemných či nepříjemných je do značné míry utvářeno na základě každodenní zkušenosti jedince v rámci konkrétní kultury, a to skrze evaluativní podmiňování a okrajově též formou pouhého vystavení se určitému pachu. Jinými slovy, že čichové preference a averze nejsou vrozené, nýbrž jsou výsledkem jejich celoživotního formování. Čichový vjem však není založen jen na čistě kvalitativní složce zprostředkované víceméně olfaktorickým nervem. Na čichovém vjemu se podílí i nerv trigeminální, jehož stimulace v jedinci vyvolává taktilní percepci pachů v nosní dutině. Oba dva tyto nervy jsou vzájemně propojeny jak na periferní, tak na centrální úrovni chemosenzorického zpracování podnětů a jejich vzájemné interakce se spolupodílí na vnímaných charakteristikách finálního pachu, tedy i jeho vnímané příjemnosti či nepříjemnosti (tj. hedonicitě).

Vzhledem k tomu, že novorozenci mají jen omezenou předchozí zkušenost s pachy, na jejich rozdílné reakci na ně by se mohla významnou měrou podílet taktilní složka čichového vjemu zprostředkovaná trigeminálním nervem. V této studii bychom tedy chtěli sledovat, jestli na pachy dráždící trigeminální nerv více a méně novorozenci reagují odlišně. Reaktivitu novorozenců jsme se rozhodli sledovat na autonomní úrovni; jmenovitě pak sledováním srdeční frekvence novorozence. Neboť u dospělých změny srdeční frekvence korelují s vnímanou příjemností pachů tak, že nepříjemné pachy vyvolávají v jedincích zrychlení srdečního rytmu. Očekáváme, že pachy dráždící trigeminální nerv více budou v novorozencích vyvolávat relativně větší zrychlení srdeční frekvence v porovnání s pachy, které trigeminální nerv dráždí méně. Tedy, že i bez předchozí zkušenosti s daným pachem bude jejich vnímaná příjemnost odlišná v závislosti na míře, kterou pachy dráždí trigeminální nerv.

Abychom otestovali tuto hypotézu, vystavili jsme padesát novorozenců (26 M) — dva až tři dny starých — tři pachovým podnětům v randomizovaném pořadí; jeden pach dráždící trigeminální nerv relativně více, jeden méně a jeden podnět, který byl bez pachové stopy. Každému novorozenci jsme ve třech po sobě následujících cyklech přiložili pod nos tyto tři podněty, během čehož jsme sledovali pomocí elektrokardiogramu jejich srdeční frekvenci. Každý cyklus byl následně

analyticky zpracován ANOVOU s opakovanými měřeními, kde meziskupinovým efektem bylo osm průměrů srdečních frekvencí, každý za deset sekund, vnitroskupinovým efektem pak pachový podnět (dráždící trigeminální nerv více/méně a podnět bez pachové stopy) a pohlaví novorozence, a základní hodnota srdeční frekvence zprůměrovaná za osmdesát sekund jako kovariáta. Analýza zjistila signifikantní interakci pachového podnětu a srdeční frekvence u prvního cyklu. Dále pak plánované kontrasty ukázaly signifikantní rozdíl mezi pachovým podnětem a opakovanými měřeními srdeční frekvence asi půl minuty po prezentaci podnětu. V dalších dvou cyklech již signifikantní rozdíly nalezeny nebyly.

Výsledky této studie ukazují na asymetrické zpracování pachů novorozenci na úrovni jejich srdeční frekvence v závislosti na míře, jakou pachy dráždí trigeminální nerv. Tato asymetrie ve vnímání a zpracování pachových podnětů by mohla hrát roli v utváření vnímané příjemnosti nebo nepříjemnosti daného pachu. Avšak naše výsledky nepotvrzují hypotézu, že by pachy dráždící trigeminální nerv více vyvolávaly zrychlení srdečního tepu.

Klíčová slova:

Chemorecepce, Chemosteze, Čich, Čichový nerv, Trigeminální nerv, Srdeční rytmus, Novorozenci, Hedonicita.

“All our knowledge begins with the senses, proceeds thence to the understanding, and ends with reason.”

(Kant, 2007, p. 242)

CONTENTS

INTRODUCTION

For years, researches have been trying to come up with a classification system that would predict how are certain odorants perceived by humans. This has proven to be a considerable challenge as several factors are influencing the perception of odours. These are based not only on the physicochemical properties of the stimuli presented, the general physiological features of the human sensory system, but also on the context-dependent cognitive modulation. It depends largely on exposure to the odours and, even more importantly, on the context in which the odour is encountered (for review see Kaeppler & Mueller, 2013), which give rise to two main processes that are thought to be forming, to a certain degree idiosyncratic, perceived hedonicity of odours.

However, recent studies show that certain odours are perceived by an individual to be more unpleasant than others with little prior exposure to them. The degree to which odorant molecules stimulate both the olfactory and trigeminal nerve seems to account for a considerable part of such variation. Together they partake on the elicit chemosensory perception[1] of odours, with the olfactory nerve providing the "pure" percept of an odorant while the trigeminal system mediates the tactile-like confound of the odorant sensation. More precisely, the trigeminal chemoreception evokes chemesthetic[2] sensations (i.e., thermal, tactile-like, and even nociceptive stimulation that depends on the degree of nasal pungency of the given odorant), all of which could activate protective behaviour designed to deter the individual from sources causing a potential health hazard to him (Cometto-Muñiz, 1998).

Understandably, complete unfamiliarity to a certain odour cannot be ensured in humans, as humans are exposed to chemosensory stimuli on a daily basis since they are born; in fact, even prenatally, for nasal chemoreceptors are fully functioning

[1] Chemosensory perception: relating to the perception of chemical signals elicit by the olfactory, trigeminal and gustatory nerves' chemoreceptors. Here, in this thesis, we will deal with olfactory and trigeminal precepts and disregard, if possible, the gustotary ones.
[2] Chemesthesis: the chemical sensibility of the skin and mucous membranes. Chemesthetic sensations arise when chemical compounds activate receptors associated with other senses that mediate pain, touch, and thermal perception. Trigeminal chemesthesis includes nasal and oral irritation, causing sensations such as tickling, burning, warming, cooling, and stinging, which could elicit reactions such as crying, sneezing, and coughing (Alarie, 1966).

in utero, far in advance of any of the other sensory receptors (Bremner, Lewkowicz, & Spence, 2012). Regardless, newborns are the ones that still could be expected to have at least very limited previous exposure to various odours, which would interact with the way these odours are perceived by them.

Our study is among those that take advantage of this fact. Here, building on the design of the few studies exploring such relationships, we decided to test whether there is a difference in occurrence of negatively-valenced facial actions and autonomic responsiveness upon presentation of unfamiliar odorants with contrasted trigeminal component (relatively strong vs. relatively mild). For, if the olfactory contribution alone does not easily predict neonatal odour hedonics, due to newborns' limited previous exposure to chemosensory inputs, one may hypothesize that together with the strength of the trigeminal contribution they may form a significant factor affecting neonatal appetitive/aversive responses to odours.

However, though our study was aimed at analysing the response elicited in newborns on the whole — both from the behaviour and autonomic point of view — this thesis analyses and reports only part of the data collected; specifically, the heart rate frequency. Thus, although in the experimental section of this thesis, the procedure of the data collection is described in its entirety, including parts not relevant to the following analysis and results, the theoretical section of this thesis is tailored only to this analysed aspect of the newborns' responsiveness; i.e., the facial and behavioural responsiveness indicative of an appetitive/aversive response is not discussed in the theoretical section of this thesis.

Instead, the theoretical section of the thesis describes the details of inner workings of the chemosensory perception provided by the olfactory and trigeminal nerves in humans in general, describing the anatomy and morphology of relevant structures and factors which could influence the odour perception and the effects odours can have on human autonomic nervous system. It ends with a chapter dedicated to viewing these aforementioned structures and processes within the perinatal paradigm, discussing the differences, similarities, and unique specifics of this developmental period.

The experimental section of this thesis is subdivided into two main parts. In the first one, two pilot studies are depicted, both with the sole aim to select stimuli necessary for Main Study in question. The necessity of careful selection of odorants presented to newborns and, more importantly, of their concentrations will become apparent in the theoretical section of this thesis. In the second part of the experimental section, Main Study is brought to focus, restating the aims of our study, while paying more attention to the intricacies of a chosen topic, design, and not to mention participants sample. This whole thesis ends with a discussion about our findings, conclusion, and implication for future research.

THEORETICAL SECTION

1. ANATOMY OF HUMAN CHEMOSENSORY PERCEPTION

The first chapter of this thesis is dedicated to discussing the anatomy of the distinct organs partaking in the human chemosensory perception (olfactory and trigeminal only) with a very brief digression into comparative anatomy, physiology, and phylogeny of olfaction and trigeminal chemesthesis.

Divided into two main parts — peripheral pathway and central pathway — this chapter follows the signalling pathway of chemosensation from the inhaled air rich with odorants, to them being detected by the olfactory or trigeminal receptors, and ending with the chemical signals this initiates and that are transformed into electrical signals into the brain only to be there perceived as smells. Their perception will be discussed in the following chapter.

In the last part of this chapter, evolutionary changes in anatomy of human chemosensory organs are discussed, focussing namely on the changes in ethmoid bone structure which led to alternation in localisation of olfactory mucosa and the appearance of partitioning between the olfactory cleft and the ethmoid labyrinth that connected the once separated respiratory and olfactory noses in humans.

1.1. Peripheral Pathway

1.1.1. Nose and Nasal Cavity

The structure of the nose, along with its cavity, is designed partly so that the inspired air flow is directed towards the olfactory epithelium. The nasal septum, a central partition, divides the nose and its cavity into two, more or less identical nasal passages, with each lateral nasal wall formed by up to four bony outgrowths, also known as turbinates, three — inferior, middle and superior — being the norm that is

in some people accompanied with the fourth one, called supreme. Nasal passages end with the nasal roof where endowed with the olfactory mucosa the olfactory fossa is situated; a slight depression in the anterior cranial cavity whose floor is the cribriform plate of the ethmoid bone.

Inspired air enters the nose through each nostril, going up the nasal passages it encounters the nasal valve area — the narrowest portion of the nasal passages — where the highest point of the respiratory tract resistance is reached. Even very small deformities of the valve area may severely impair the dynamics of nasal air flow (Patel & Pinto, 2013). Alternation of this laminar airflow could result in turbulences, which could affect the direction of air superiorly directed towards the olfactory epithelium (Zhao, 2004) where the detection of odorants starts and thus impair the sense of smell.

The inspired airflow enabling the transport of volatile molecules to the olfactory mucosa is only one condition for the successful detection of odorants which is one of the several function of the nose, others include humidification and warming of air before its arrival in the lower airway by the turbinates. Humidity of the inspired air also plays an important role as the olfactory mucosa needs to be watery enough for the volatile chemical molecules require aqueous mucus to be dissolved in. Once dissolved, they are transported to the olfactory receptors located on the ciliated olfactory epithelium buried deep in the mucus layer with the help of chaperones, also known as odorant-binding proteins. These chaperons, while carrying the dissolved odorant on their surfaces, not only help to ease its way to its receptor, but also assist in the successful binding of the odorant to its olfactory receptor, a process which induces signalling and thus facilitates olfaction (Hornung & Mozell, 1981).

Under normal resting breath condition, it has been estimated that only 10% of inhaled air will actually reach this olfactory region, the rest escapes between the inferior and middle turbinate along the lateral nasal wall (Hahn et al., 1993). The percentage of inhaled air that reaches the olfactory epithelium could be enhanced in sniffing (i.e. the active process of smelling) (Wachowiak, 2011). Both, physiological breathing and sniffing, play a role in the so-called orthonasal olfaction where the inspired air goes through the nostrils, over the olfactory receptors, and then directly

to the lungs. The other form of olfaction is retronasal. Its passage differs in that the air laden with volatile odorants' molecules encounters the olfactory epithelium via the nasopharynx during eating and drinking. For the air to enter the nasal cavity this way, the velopharyngeal flap must be open. Once in the nasal cavity, similar to the orthonasal olfaction, the odorants dissolved in the mucosa bond with their olfactory receptors (Stevenson, 2013).

1.2. Central Pathways

Once the successful binding of the odorant is achieved, the neural signalling can begin, leading to chemosensory percept. Chemosensation in the nose is located mainly in the olfactory epithelium and is mediated by two cranial nerves; the olfactory nerve and trigeminal nerve.

1.2.1. Olfactory Epithelium

The olfactory mucosa is the part of the nasal mucosa that plays a key role in olfaction. The human olfactory mucosa appears slightly yellow and without a distinctive hue as seen in, for example, rodents (Kachramanoglou et al., 2014). The olfactory epithelium constitutes of four cell types:

- basal cells: stem cells of the olfactory epithelium resting on the basement membrane, acting as the potential sources of neuronal regeneration,
- ciliated olfactory receptors: bipolar neurons forming the olfactory nerve,
- sustentacular cells: supporting cells surrounding olfactory receptor neurons,
- microvillar cells: hypothesized to act as a second morphologically distinct class of chemoreceptors (Escada, Lima, & Madeira da Silva, 2009).

In humans, it is mostly located on a very small portion of the ethmoid labyrinth, called the olfactory fossa, overlying the superior nasal septum, the cribriform plate, and the upper part of the superior turbinate. The size of olfactory epithelium is directly tied to the olfactory sensitivity for the olfactory receptors are located here. Human olfactory mucosa covers a more or less dime-sized region accounting approximately for only 3% of the nasal cavity surface area (Chamanza and Wright,

2015), with about 10 million olfactory cells; each of which has about 350 different receptors specific for only one odorant type.

It is worth noting that the findings of several recent studies have pointed to a more extensive distribution of olfactory epithelium, showing that it not uniquely bound to the olfactory fossa but could, in fact, extend farther both in the anterior or posterior direction (Leopold et al., 2000; Féron et al., 1998). Moreover, the location of the olfactory epithelium is highly variable both on the inter- and intrapersonal level, the main factors that bear influence on this are the environmental conditions of the said person, his or her age, and health status. Interestingly enough, it could be also used as an early marker of neurodegenerative conditions, such as schizophrenia, Alzheimer's disease, multiple sclerosis, and Parkinson's disease (Escada, Lima, & Madeira da Silva, 2009).

1.2.2. Olfactory Nerve

The olfactory nerve is the first cranial nerve; it is considered to be the shortest of the twelve cranial nerves and, along with the optic nerve, does not emanate from the brainstem. The olfactory nerve starts in the olfactory epithelium as the ciliated bipolar olfactory receptor cells; each receptor cell with a single dendrite endowed with a knob-bearing olfactory cilia having 10 to 20 cilia. As a true bipolar neuron; each bipolar olfactory receptor cell ends yet again with a single unmyelinated axon leading up, joined with others into fascicles and nerves, through the many openings of the cribriform plate of the ethmoid bone to synapse within the olfactory bulb (Escada, Lima, & Madeira da Silva, 2009). The olfactory bulbs are located on the both inferior sides of the brain, under the ventral surface of the frontal lobes (Kratskin & Belluzzi, 2003). They consist of six layers:

- Olfactory nerve layer, the outermost layer of the bulb,
- Glomerular layer where the neurons from the olfactory epithelium branch and synapse to the dendrites of principal and intrinsic cells (i.e., the second-order projection neurons),
- External plexiform layer consisting of a dense network of synapses between dendrites of principal neurones and granule cells,

- Mitral cell layer, a place of synaptic processing based on the excitatory (from olfactory sensory neurons) and inhibitory (from granule cells) input,
- Internal plexiform layer, a very thin layer full of axons of second-order projection neurons,
- Granule cell layer, the innermost layer of the bulb containing migrating immature interneurons (Zou, Chesler, & Firestein, 2009).

In the olfactory bulbs, the olfactory information is coded and then sent along the olfactory nerve to be further processed in the "primary olfactory regions" (piriform cortex, olfactory nucleus and tubercle, and amygdala) and the "secondary olfactory areas" (hippocampus, hypothalamus, thalamus, orbitofrontal cortex, and the cerebellum) (Pinto, 2011).

1.2.3. Trigeminal Nerve

The trigeminal nerve, on the other hand, is the largest among the cranial nerves, with not only sensory function — tactile, proprioceptive, and nociceptive afference to the face and mouth — but also motor function — innervating several facial muscles and facilitating movements such as biting and chewing.

There are three branches of this nerve, thus its name, the ophthalmic nerve, the maxillary nerve, and the mandibular nerve. The first two are purely sensory, the third one provides also the motor functions. Free endings of the ophthalmic and maxillary nerve, located in the epithelium of the nasal vestibule and nasal chambers, are sensitive to most volatile molecules as well.

Stimulation of these afferent axons travels up to the trigeminal ganglion, located in the Meckel's cave just over the ear canal, where the three major branches of the trigeminal nerve converge. The sensory root relays signals to the ventral posterior medial nucleus of the thalamus and then to the cortical areas that process facial irritation and pain (Pinto, 2011).

1.2.4. Vomeronasal Organ

The existence and function of the vomeronasal organ in humans remains an open question. In many animals, the vomeronasal organ, also known as the Jacobson's organ, constitutes an accessory olfactory organ that receives certain, biologically important chemical stimuli (e.g., pheromones). Most studies asserted that in humans, it disappears during the prenatal development and remains present only in few individuals in the form of the vomeronasal epithelium located in the anterior nasal septum (for review see Witt & Hummel, 2006).

Thus, in humans, vomeronasal organ could be considered an atavism (i.e., a structure that is not commonly observed in individuals nowadays but was ubiquitous in members of their evolutionary lineage) and therefore, one of the several evolutionary changes of human olfactory organs (Smith, Laitman, & Bhatnagar, 2014). In recent years, however, some studies have proven the still existing connection between its nerves and the hypothalamus and the terminal nerve and are even pointing to a possible endocrine function of the vomeronasal organ in adult humans (Wessels, Hoogland, & Vorster, 2014).

1.3. Evolutionary Changes

The olfactory brain proportion of total brain volume has decreased from 0.01 in prosimians to 0.001 in monkeys to 0.0008 in great apes to 0.00009 in humans (King & Fobes, 1974). Moreover, it is known that the olfactory epithelium and the olfactory bulb in primates are proportionately smaller than those in most other mammals (Pihlstrom et al., 2005). These reduction of volumes of the regions of the nasal cavity that are covered with olfactory epithelium, the olfactory bulb, and piriform cortex, were the reasons for why humans, along with other primates, were designated to be "microsmatic" (i.e., more reliant on the visual input with a reduced importance of olfactory information) as opposed to the "macrosmatic" species, mostly carnivores with exceptional sense of smell (Smith, Bhatnagar, Tuladhar, & Burrows, 2004).

There are several hypotheses, based on evolutionary models, which argue that in humans, along with other primates, the sense of olfaction could have

regressed as we became more dependent on other senses. Even if compared with the other primates, humans seem to be still less reliant on the chemosensory input (Niimura, 2009). A trade-off between the visual and olfactory modality has been suggested as one of the reasons as it does, to a certain degree, correlate with the early emergence of colour vision and more importantly the later one of trichromatic vision in primate evolution (King & Fobes, 1974).

One of these hypothesis is based on the bipedal locomotion of humans, which freed the hand and increased to role of vision, both of which gave rise to the meaning to the sight and touch, while, at the same time, distancing our olfactory passages from the odour-rich floors. These might have been the causes for olfaction not being that important for humans and thus leading to the physiological changes — observed not only in humans but other primates, apes, and monkeys as well — such as the retraction of the snout and anterior migration of the orbits. These primates also possess olfactory organs which are smaller in relation to their skull size.

No matter the reasons, there are some meaningful physiological changes in olfactory anatomy unique to humans. One of them is the modification of ethmoid bones morphological structure. Ethmoid bone is considered to be one of the highest phylogenetically conserved region among the cranial and facial bones (Hanken and Thorogood, 1993), and its area has been showed to be directly proportional to the area of the olfactory epithelium in many of the mammalian species, humans included (Pihlstrom et al., 2005). In humans, part of it evolved into the intricately structured ethmoid labyrinth, consisting of a number of thin-walled cellular cavities arranged in three groups. It is currently considered to act as sinuses involved only in housing and protecting the olfactory function (Jankowski, 2011) because most of its surfaces are lacking olfactory mucosa; only a very small portion of ethmoid labyrinth, called the olfactory fossa, is enhanced with this mucosa. Moreover, its remodelling led to the dissipation of the transverse lamina, and thus, a connection of the respiratory and olfactory noses in humans was established.

Nevertheless, irrespective of the several changes occurring in human olfactory anatomy, in recent years it became apparent that humans rely heavily on olfactory cues in several aspects of their everyday lives. With that even the previously used terminology that divided species into “microsmatic” and

"macrosmatic" categories fall under scrutiny. These terms were defined solely morphologically — based on the volume of olfactory bulb and olfactory mucosa — and even though, as stated above, the comparative size of both could, to a certain degree, predict the effectiveness of the olfactory modality, there are other, more important aspects predicting such a skill, to name just a few the thickness of the olfactory mucosa — its cilliation could enhance the surface area quite considerably — and the number of olfactory receptors expressed (Smith, Bhatnagar, Tuladhar, & Burrows, 2004).

Thus, the following chapter will discuss the olfactory perception in humans, underlining the importance this sense still possesses in our species in several aspects of our everyday lives.

2. CHEMOSENSORY PERCEPTION

As discussed in the previous chapter, olfactory perception begins with the air being transported to the olfactory epithelium where the receptors, covered in mucosa, lie waiting to be triggered. Though the number of functional olfactory receptors is limited in humans, they are not odorant specific; some odorants are recognized by a number of receptors and, vice versa, some receptors are triggered by a number of odorants. It seems that there is a combinatorial receptor coding scheme to human olfaction (Malnic, Hirono, Sato, & Buck, 1999) where which receptors gets triggered is influenced not only by their chemical structure (e.g., odorant's solubility in water could affect the speed with which they reach the receptor), but also the concentration of odorants.

This combinatorial receptor coding scheme could also help to explain why there seems to be a spatial and temporal presentation of the perceived odorant in the brain. For if an odorant stimulates several olfactory receptors at once, and vice versa, this combinatorial stimulation is likely to lead to a distinctive zone pattern of activation across the glomerular layer of the olfactory bulb, creating a so-called sensory map in the olfactory bulb (Ressler, Sullivan, & Buck, 1993). This zonal patterning might be the information which is transmitted down the olfactory nerve to the "primary olfactory regions", namely to the piriform cortex, thus leading up to the olfactory sensory information being decoded, coded, and stored in the brain.

The coding of the olfactory sensory information in the brain and, more importantly, its storing, both of which influences the olfactory related cognition, is influenced by other sensory inputs as well, thus helping to construct the biologically important meaning of the information needed to be processed. Therefore, it is common for one modality' response to be influenced from the input coming from another one. Often times, this is an enhanced — sometimes even supra-additive — response to information from one sensory system due to the brain receiving parallel input from another modality (Prescott, 2012).

The highly developed skill of lip reading in the hard of hearing population could be an example of a process where one modality might compensate for the non-

existence of another. As far as the influence among senses go, the so-called McGurk effect (McGurk & MacDonald, 1976) could be used as an example of a perceptual phenomenon that demonstrates an interaction between hearing and vision in speech perception and its potential influence on phonetic processing. Similar evidence is abundant for smell–taste interactions; in cases of a common cold, the flavour of the perceived food is frequently absent due to changes in the nasal air-flow or to the consistence of olfactory mucus preventing olfactory receptors from being triggered, and we are left to rely mostly on our taste buds, covering only the five primary tastes — salty, sweet, sour, bitter, and umami. The flavour of odorants could be also affected by the way they are presented, with changes in detection and perceived quality of an odorant depending on the way they are presented, i.e., orthonasally or retronasally (Burdach, Kroeze, & Köster, 1984). Another way the perceived flavour could be influenced is by the visual input, for it has been shown that the flavour of a beverage is influenced not only by the colour of the drink itself (Jackson, 2002), but also by the colour of the ambient lighting of the surroundings (Oberfeld et al., 2009).

To reflect this very close multimodal integration, this chapter is called chemosensory perception; a cognitive construct derived from the close synthesis of gustatory, olfactory, and trigeminal inputs, all of which are making organisms capable to closely monitor the chemical environment surrounding them. Namely, this chapter will be focusing on the olfactory and trigeminal sensory input into the odorant perception as these are very closely intertwined. We do omit the gustatory one; the reason being that in this thesis, we wanted to include only the information of the utmost relevance to the executed study and, as in this study, we chose an orthonasal way of presenting the stimuli, we felt that the effects of taste receptors on the newborns' responsivity could be disregarded for their potential influence would be minuscule.

As regards the trigeminal part of odorant perception, its process is yet to be understood. In recent years, several trigeminal receptors were identified, proven once and for all that the chemo-activation of trigeminal system is not, by any means, as nonspecific as used to be believed; to name just the most famous one, the discovery of capsaicin receptor eliciting the sensation of burning pain when eating chilli peppers (Tominaga et al., 1999). Still, contrary to olfactory perception, very little is known about the binding and stimulation of trigeminal receptors to certain odorants

(Frasnelli, Albrecht, Bryant, & Lundström, 2011) not to mention the way trigeminal activation interacts with the olfactory sensory input to elicit the specific behavioural and cognitive response to each perceived odour.

Because of its close interconnection to olfactory perception and because many odorants, especially at high enough concentrations, stimulate not only the olfactory nerve, but also the trigeminal one (Doty, 1995), main findings regarding this phenomenon in humans would have to be done, and are done, on congenital anosmics[3], a condition so very rare and in isolated cases often undiagnosed that only few studies were, in fact, conducted. Laska et al (1997) found that congenital anosmics with no olfactory percept could, based on purely the stimulation of trigeminal free endings, effectively discriminate the odorants presented, a skill that would point out to a possible qualitative assessment of the odorant via solely trigeminal stimulation. As stimulation of the free endings of the ophthalmic and maxillary nerve mainly results in sensations best described using physically or thermally tangible descriptors such as irritating, tingling, stinging, burning, cooling, painful, or pungent (Alimohammadi & Silver, 2015), the term chemesthesis is often employed to express the ability of trigeminal nerve endings to provide tactile-like sensation to chemical stimulation (Cain et al., 2006).

Another difference between the trigeminal and olfactory perception in humans is the ability to localize which nostril is being presented with the odorant. In 'relatively pure' odorants — i.e., odorants which mostly activate the olfactory nerve no matter their concentration like for example vanillin is — monorhinal presentation does not enable the person to say, from which site the odour is coming. However, the stronger the degree to which an odorant activates the human trigeminal system, the better the detection from which nostril the odour is coming (Frasnelli, Hummel, Berg, Huang, & Doty, 2011). Thus, being able to recognise laterality of a unilaterally presented stimulus can provide grounds to measure the odorants' level of trigeminal irritation.

[3] congenital anosmia is a highly rare condition in which people are born with a inability to smell. It may be syndromic with a with a specific genetic disorder diagnosis (such as Kallmann syndrome and congenital insensitivity to pain) or isolated with no additional symptoms and genetic origin not yet understood (Karstensen & Tommerup, 2011).

2.1. Measurements of chemosensory perception

Measurements of chemosensory perception could be psychophysical — based upon a behavioural response — physiological — often including some type of biomedical instrumentation — or some combination thereof.

2.1.1. Physiological measures

Physiological methods are either based on electrophysiological measurements of olfactory related organs, which would include the measuring of negative mucosal potentials in the nasal cavity or of chemosensory event-related potentials (CSERPs) by averaging electroencephalographic signals occurring in relation to nasal stimuli. Others could be based on functional and structural imaging of relevant organs; e.g., by employing fMRI to monitor and distinguish cortical activity patterns related to trigeminal stimulation (‘chemosomatosensory evoked potentials’) from those elicit by olfactory stimulation (‘olfactory evoked potentials’) (Hummel, 2000).

These methods employing neuroimaging procedures are not applicable for newborns, however, in recent years near-infrared spectroscopy has been used on newborns, even premature ones, to monitor the changes of cerebral blood flow in the olfactory cortex (Bartocci et al., 2001).

Furthermore, the orbital muscle response could be tracked down, along with the quantification of nasal secretions or nasal lavage markers. Moreover, rhinomanometry, laser-Doppler flowmetry mucociliary clearance tests could be performed. Not to mention that the respiratory patterns are often recorded to measure the breathing frequency and depth (Shusterman, 2002).

2.1.2. Psychophysical (perceptual) measures

A psychophysical test procedure is "[a]ny procedure that provides a quantitative measure of sensory function and requires a verbal or conscious overt response on the part of the examinee" (Doty, 2015, p.230). These procedures are mostly employed to measure the main olfactory tasks (detection, discrimination, and identification of an odorant).

Testing the detection of threshold sensitivity is fundamental in understanding the characteristics of chemosensory perception — threshold concertation being the lowest detectable concentration of an odorant. In these test, the olfactory and trigeminal perception of a given odorant proves to be vital as the threshold level of a qualitative sensation of an odorant emerges usually at much lower concentration than the threshold level of its chemesthetic sensation (Cometto-Muñiz, 1998), both follow a sigmoid function but the function of chemesthetic detectability appears to be considerably steeper than the olfactory one (Cometto-Muñiz & Abraham, 2015).

Other tests include, odorant recognition test with or without provided contextual cues or quality discrimination tests, where a participant is asked to either numerically rate the perceived similarity of sets of odorants, choose the odd one from triadic comparisons, or express their perceived similarity or dissimilarity using a scale.

Aforementioned tests are influenced by several factors, both from the participant's and the odorant's side as well. These will be discussed in the following chapter as the relationships between them are highly interconnected, sometimes even intertwined to a degree indiscernible to existing methods of measurements.

3. PERCEIVED QUALITY OF ODOURS

The input from the olfactory and trigeminal nerve activates areas in the primary and secondary olfactory regions, many of which are parts of the limbic system; a complex set of brain structures responsible, among other things, for the formation and storage of memories associated with emotional events, motivation, and reward system (Pinto, 2011). Stimulation of these creates and modulates the perceived quality of an odorant (i.e., judgments of its familiarity, intensity, and hedonicity) in the human life (Delplanque et al., 2008).

In this chapter, we look at the various factors influencing perceptual characteristics of odours, especially hedonics. These include physicochemical properties of the odorant itself, the inter- and intrapersonal variation in humans, and contextual factors such as repeated exposure or experimental instructions (Rouby et al.,2009). As discussed in the introduction to this thesis, though these aspects play a key role in this life-long process of induction and formation of chemosensory hedonics in the person's life, the design of our study was conducted so that these aspects could have been disregarded in the analysis performed as they could be expected not to play a relevant role. Yet, we do acknowledge this to be an oversimplification on our part and realise how immensely interconnected all these factors are. For example, the odorant's perceived intensity is driven by its concentration which could also affect the perceived pleasantness of the said odorant. The perceived pleasantness is related to the odorant's chemical structure (Khan et al.,2007) as well as to the individual's physiological and cognitive factors (e.g., Rouby et al.,2009), and other perceptual characteristics, such as perceived intensity, and thus again the odorant concentration (Doty,2015).

The perceived intensity and actual concentration of an odorant is important, as stimulus intensity does seem to affect the perceived odour pleasantness; a shift in one dimension (intensity of an odorant) is often accompanied by a shift in the other (perceived quality of an odorant) and vice versa as with the increased odorant's concentration other receptors could be activated, creating a concentration specific perception of an odorant (Laing, 2003).

Another factor that needs to be taken into account is the judgment of the odorants familiarity where the frequency and more importantly the contextual link created with that said odour seems to be of significance (Delplanque et al., 2008). The design of our study was created in an effort to, by employing newborns with little or no prior experience with stimuli presented, eliminate these factors of the perceived quality of an odorant and thus, focus mainly on the physicochemical feature of the presented stimuli as a factor that influences the perceived quality of an odorant, more importantly on their varying level of trigeminal pungency.

As in our study, only dilutions of a single chemical compound in an appropriate diluent were used as stimuli, we also took the liberty to omit the discussion as of perception of odorant mixtures. The odorants in the mixtures could be perceived as homogeneous — blending into one, creating a new odour recognized by an individual as one entity — or heterogeneous with at least some of the odorants still distinguishable in the mixture (Thomas-Danguin et al., 2014).

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3.1. Odorant based factors influencing its perception

The relationship between physicochemical features of odorants and their perceived odour quality is an important issue in olfaction, and although most studies agree that the molecular structure of odorants determines, to a certain degree, their perceived odour, the exact nature of this relationship remains unknown as other factors play an important role in creating this relationship. As discussed previously, the olfactory and trigeminal nerves are activated by successful binding of an odorant to its receptor. This binding, and thus recognition of an odorant, depends on the molecular structure of the odorant and its receptor, be it olfactory or trigeminal one. For example, it has been shown that the length and branching of the carbon chain and the type of functional group were critical for activating an olfactory receptor specific for aldehydes (Firestein, Araneda, & Kini, 2000).

In some cases, it is so specific that it may be possible to predict a neuronal activation solely on the basis of the odorant chemical structure and thus allowing us to predict hedonicity of a novel odorant based solely on its physicochemical properties (Khan, 2007).

3.2. Inter and intrapersonal variation of odour perception

Odorant perception varies greatly both on the inter- and also intrapersonal level. Our response to an odour may differ throughout the day, be dependent on our emotional state and satiation level (Soussignan, Schaal, & Marlier, 1999), or in women, vary across their menstrual cycle (for review see Martinec Nováková et al., 2014).

Other factors that could influence the odorant perception are:

- **Gender.** Women, regardless of their ethnicity and genetics, are superior to men in their ability to identify and perceive odours (for review see Brand & Millot, 2001), and they also tend to be more 'sensitive' as far as chemesthetic stimulations go, detecting irritants at lower concentration with a stronger physiological reaction to them (Cometto-Munitz & Noriega, 1985). However, these gender specific variations in reaction occur in the secondary olfactory areas (namely amygdala), no significant differences in the olfactory bulb activation were detected and thus, it could be argued that these differences could be ascribed to higher cognitive function and not to chemosensitivity per se (Radulescu & Mujica-Parodi, 2013).

- **Genetics.** Some ethnic backgrounds are associated with differing odour detection abilities and thresholds (Doty, Applebaum, Zusho, & Settle, 1985). In studies on twins, monozygotic twins display more similarity on olfactory thresholds than the dizygotic ones (Segal, Brown, & Topolski 1992).

- **Age.** Increasing age is correlated with higher odour detection thresholds (lower sensitivity) in adults (Cain & Gent, 1991) till the age of 60, after which the decrease in sense of smell becomes even more apparent, leaving many over the age of 80 with a demonstrable smell loss (for review see Doty & Kamath, 2014). In chemesthetic experiments, age has proven to yield conflicting results and more studies need to done to examine this interindividual variable (for review see Shusterman, 2002). Aging also influences the perceived odorant hedonicity. Pleasantness of odours perceived on average as unpleasant does not seem to show age-related changes (Konstantinidis, Hummel, & Larsson, 2006).

- **Medical conditions**. A variety of medical conditions have been associated with several deficits and dysfunction in olfaction, some of neurodegenerative origin, like Alzheimer's or Parkinson's disease (Doty & Kamath, 2014), some, like ADHD

or schizophrenia, connected to mental health. There the exact pathophysiology remains yet to be understood for it may seem that at least some observed deficits are to be ascribed to the treatments administered (Romanos et al., 2008; Brewer et al., 2001).

- **Alcoholism and smoking**. Both of them show dose-dependent impairment in olfactory perception, sometimes of long-term nature where after the cessation of the substance abuse the olfactory perception was not recovered (Ditraglia et al., 1991), and, at least in case of heavy smokers, a significant decrease in nasal irritant sensivity is found, which, again, could be ascribed to pathophysiological changes (e.g., the thickening of olfactory mucosa) occurring in heavy smokers (Cometto-Munitz & Cain, 1982).

- **Occupational and environmental factors.** Repeated inhalation of various chemicals could cause damage to the olfactory epithelium (Antunes, Bowler, & Doty, 2007).

3.3. Contextual factors influencing odour perception

Apart from these factors, based on the stimuli presented and on the perceiver, the external conditions in which the odorant is presented could influence its perception as well. We already discussed the effect of the surroundings (Oberfeld et al., 2009) and the manner in which the stimuli is presented (Jackson, 2002) having an effect on its perception. However, there are other contextual factors important in hedonic processing of odours.

The perception of odorant could vary and be modulated in relation to the previous stimuli (Cain et al., 1978) and be improved by repeated exposure to that same stimuli, known as the mere exposure phenomenon (Delplanque et al., 2015). Furthermore, the personality and current emotional state of the individual could have an effect on their odour pleasantness judgements (Chen, 2005).

Positive or negative verbal labelling of odours is also known to cause shifts in odorants' perception in adults as well as in children (Bensafi et al., 2007) and even lead to differential processing on the neuronal level (de Araujo et al., 2005).

4. THE AUTONOMIC NERVOUS SYSTEM RESPONSE TO ODOURS

As we have seen in the previous chapter, there are several factors influencing the perceived odorant hedonicity. Pleasant and unpleasant odours activate different respective neural networks (Zald & Pardo, 1997) leading to a differential response. In fact, the experienced pleasantness or unpleasantness of odorants could influence both our behavioural and autonomic responses. These are measured by parameters such as skin potential, skin resistance, skin temperature, skin blood flow, respiratory frequency, and heart rate.

The autonomic nervous system which regulates the involuntary control of smooth, cardiac muscles and glands, controls the innards of the body and is responsible for maintaining homeostasis. Its neurons carry information about the inside of the body to the brain —preferentially to hypothalamus, pons, brainstem, and medulla oblongata— and thus the automatic and continuous regulation of internal organs — including those of the digestive, endocrine and cardiac system — is achieved (Shields, 1993). The autonomic nervous system is divided into the sympathetic nervous system and parasympathetic branches; sympathetic nervous system is activated in emergencies, eliciting the so called “flight-or-fight” reactions while parasympathetic nervous system works in opposition by creating the “rest-and-digest” reactions, together they are responsible for the dynamic balance of homeostasis by maintaining the body's internal environment (e.g., temperature, oxygen level, and salt concentration). The autonomic nervous system also plays an important role in emotional experience as experienced — for example — by blood pressure and heart beat changes (for review see Kreibig, 2010).

Perception of odours, especially their perceived pleasantness or unpleasantness, is known to elicit affective states, both negative and positive ones, even leading to a olfaction-induced emotion (Alaoui-Ismaïli et al., 1997) with unpleasant odours eliciting disgust and anger. These olfaction-induced emotions were perceived on autonomic level as well.

Several studies have shown that an exposition to an unpleasant odour could produce a defence response of the autonomic system — heart rate acceleration — avoidance tendency and behaviour that turns the individual away from this source of stimulation in order to minimise the intake of the odour. Analogically, exposition to a pleasant odour lead to a heart rate decrease (Alaoui-Ismaïli et al., 1997; Bensafi et al., 2002a; Delplanque et al., 2009). Heart rate variation do not occur when judgements of familiarity are required (Bensafi et al., 2002b).

Therefore, heart rate variations seem to be a relevant physiological indicator of perceived pleasantness or unpleasantness of an odour noticeable rather quickly on the involuntary, autonomic level with unpleasantly perceived odours leading to a heart acceleration and vice versa odours that are perceived as pleasant decrease the heart rate. In our study, we decided to use the heart rate variation as an indicator of newborns responsivity to odours stimulating trigeminal nerve more, those, we hypothesise, would lead to heart rate acceleration (Tuladhar et al., 2005) indicative of arousal magnitude of the autonomic nervous system.

5. PERINATAL CHEMOSENSATION

This last chapter of the theoretical part of this thesis will be discussing developmental changes in anatomy and physiology of humans with the sole focus on the antenatal and perinatal period. Such a narrow focus was chosen as newborns are the participants of our study. Very few studies have been done on them, for obvious reasons of ethical and practical limitations of working with newborns — general difficulty and unpredictableness of their behaviour; their cooperation cannot be expected and/or ensured. Thus, very little is known about their responsiveness to chemosensory stimulation and often times neonates of other species are involved as approximate models with varying level of reliability (Schaal, 2004).

Reflecting the structure of the previous parts of this thesis, here we discuss anatomy and physiology of newborn's olfaction, paying a special attention to certain developmental stages and their chemosensory perception. With the main aim of this thesis in mind, we have concentrated on the perceived quality of dilutions of a single chemical compound in an appropriate diluent; furthermore, as discussed before, the perception of odorant blends is much more complicated (Thomas-Danguin et al., 2014). Thus, the biologically odours, so often used as stimuli in neonatal studies because of their immense ecological validity, will be omitted in this chapter if possible.

5.1. Perinatal anatomy and physiology of olfaction

Development of any sensory modality requires a source of stimulation. In utero, chemoreception is mediated via two main sources — placenta and amniotic fluid. Amniotic fluid, originating from maternal, fetal, and placental tissues, is the major source of this mediation as it contains many constituents, ranging from nutrients to the flavours reflecting the mother's dietary and environmental exposure (Orczyk-Pawilowicz et al., 2016). By the 10th week of gestational age, this constantly changing fluid is being inhaled and/or swallowed by the newborn in what looks like sudden bursts of movements, reflecting future breathing patterns (Andonotopo & Kurjak, 2006). Therefore, the fluid is in continuous contact with the oral and nasal

chemical receptors, creating two possible modes of sensation bind together, oral and nasal. These receptors are the same ones that could also be triggered by fragrant molecules entering the blood stream via the placenta — the second source of stimulation — a permeability of which increases with the gestational age, allowing for the transfer of odorants the mother has ingested (Schaal, 2000).

Developmentally, chemoreception (together with gustation) comes as first, only to be preceded by maturation of some somesthetic receptors. The free endings of the trigeminal nerve in the nasal cavity appear around the 4th week of gestational age and respond to stimulation by the 7th week (Müller & O'Rahilly, 2004). Ciliated neuroreceptors in the olfactory epithelium are detectable around the 1lth week. Olfactory marker protein, expression of which is related to the mature olfactory receptor neuronal activity, is detectable around the 30th gestational week, pointing to an already operating fetal olfaction (Levene, Chervenak, & Whittle, 2001).

By the third trimester of gestation, all chemosensory systems of the nose seem ready to be functional along with the morphology of nasal structures needed for the flow of the inhaled amniotic fluid to be directed at the olfactory mucosa. Thus, newborns are born with a fully morphologically and physiologically functioning nasochemoreception.

In the oral cavity, chemoreception is mediated via the trigeminal nerve and the nerves belonging to the gustatory system. The onset of taste bud formation starts around the 8th week, and by 15th weeks of gestational age, the morphologically matured taste buds are displayed all over the oral cavity, later to remain only on the tongue and on the anterior hard and soft palate (Witt & Reutter, 1996).

Another system that needs to be mentioned here, as it may, contrary to adults, play a significant role in fetal chemosensation, is the so-called accessory olfactive system connected directly to the vomeronasal organ. It starts developing around 5–8 weeks of gestational age and reaches its maximum development between 13–18 weeks after which it is assumed to be on its decline. Embryological studies show that the nervous connections with the olfactory bulb degenerates completely before birth and thus the vomeronasal organ should play no role in the human sense of smell after that (Smith & Bhatnagar, 2000). Though, as mentioned in the second chapter of this thesis, his existence and functioning in human adults remains yet to be understood as

it does appear to retain, in at least some individuals, its connection to the hypothalamus and the terminal nerve; a nerve which sends free nerve endings to the anterior nasal septum and to the olfactory mucosa and which sensory functions remains unexplored in humans no matter their developmental stage (Schaal & Orgeur 1992).

5.2. Antenatal chemosensory perception

The evidence for functioning of perinatal chemosensory perception in humans is weak and inconsistent. In utero, the easiest modality to study is the auditory as the approaches are non-invasive. For assessing the olfactory modality of human foetuses, several researchers have used indirect strategies, such as measurement of fetal response to chemical input, or newborns born prematurely are being engaged, with the late to modern stage of prematurity, or several animal models employed for approximation, from those it would appear that the fetal autonomic and behaviour responses differ in relation to qualitatively and/or quantitatively distinct odorants, which would point to prenatal ability to discriminate odours (Schaal, 1992).

Other than that, little is known about the actual, in vivo, antenatal chemosensory perception in humans as only indirect strategies to assess its function are available to us. From those, it appears that, as early as 14–16 weeks of gestation, a foetus is reacting to the dietary compounds ingested by the mother (Goldstein et al., 2003). Moreover, the odorant presumed to have predominated in utero is preferred by the neonate at least for few days after gestation (Schaal, 2000), an effect that could potentially have implications for dietary preferences of infants and children and possibly even extend its influence well into adulthood (e.g. Haller, 1999). Apart from that, to assess the chemosensory prenatal development, we have to rely on studies performed on premature newborns or on our general understanding about human physiology, from which we can assume that the prenatal prolonged exposure of chemosensory system to the stimulation may affect the newborn's development on structural, functional, and thus even behavioural level, helping even to build the perceptual ability of the said newborn (sensitivity, discrimination, and formation of preferences) (Doty, 1995).

5.3. The perceived quality of odours

As mentioned above, the moderate to late premature newborns' olfactory system appears responsive to odorants, though these responses seem more unstable than those of physiologically born newborns. Furthermore, they seem to be correlating with the neonate's gestational age, as, for instance, the neonates' responsiveness to chemosensory stimulation before the 29th week is highly unpredictable (Schaal, Hummel, & Soussignan, 2004). Furthermore, their stress responses to odorants stimulating trigeminal nerve are more pronounced; even at very weak concentrations, these odorants elicit in premature newborns rapid and shallow breathing and tachycardia (Van Reempts et al., 1997). These findings suggest that, as far as their responsiveness to odorants varying in trigeminal potency goes, prematurely born infants are generally more sensitive to trigeminal stimulation than the term-born ones are; these react in a similar way but their elicited respiratory and cardiac reaction appears to be less dramatic when compared with the preterm responsiveness (Ramet et al., 1990).

The exact opposite could be said about the olfactory stimulation, as has been shown that the premature newborns possess higher detection thresholds to odorants stimulation olfactory nerve more, reacting less to olfactory odorants both in the lowest concentrations and in the higher concentrations (Schaal, Hummel, & Soussignan, 2004) which would point to functional but less sensitive olfaction in premature newborns.

Full-term newborns olfactory system appears to function similarly to that of an adult one, contrary to their visual and auditory sensory system. The ability of newborns to detect odorants increases quite considerably over the first two days of their life and by the third day, their facial responses to pleasant and unpleasant odorants appear remarkably similar to the expressions these odorants elicit in adults (Soussignan et al., 1997), e.g., a smile suggested to be a sign of appetitive response linked with "pleasant-to-adults" odorants (Vanillin) and nose wrinkling as an expressions of aversive response linked with "unpleasant-to-adults" odorants. However, these early signs of discrimination of the hedonic properties of odorant do not necessarily match their physiological reaction (e.g., changes in their respiratory and cardiac rates) (Lipsitt, Engen, & Kaye, 1963). Hedonicity-valenced changes of

cerebral blood flow have been proven to be a more accurate indicator of cortical activation in the olfactory region, with odorants perceived as pleasant by the adult population eliciting an increase in blood oxygenation in the orbito-frontal olfactory area in newborns, unpleasant ones decreasing oxygenation in that the same region (Bartocci et al., 2000; Bartocci et al., 2001).

It should be noted though, that most of the aforementioned studies — by using stimuli both perceived as pleasant by the adult population and known not to irritate the trigeminal nerve or, on the other hand, unpleasant and possessing a high trigeminal component —could not distinguish if these observed hedonicity-valenced changes were results of the perceived olfactory pleasantness of the stimuli, or the trigeminal irritation, or a combination of thereof.

EXPERIMENTAL SECTION

1. PILOT STUDIES

Two pilot studies needed to be conducted in order to select the odorant stimuli. A careful selection of odour stimuli was essential for our odorants needed to fulfil these three following conditions.

1. Stimuli had to be proven non-harmful to humans and already used in studies performed in newborns and, if not possible, children from the lowest age range.
2. Stimuli must possess a varying level of trigeminal pungency and perceived pleasantness. Out of those odorants meeting the first condition, after assessing their levels of trigeminal pungency and perceived pleasantness in adult population, eight odorants were selected thusly:
    - 2 odorants perceived as unpleasant with relatively strong trigeminal pungency,
    - 2 odorants perceived as pleasant with relatively strong trigeminal pungency,
    - 2 odorants perceived as unpleasant with relatively mild trigeminal pungency,
    - 2 odorants perceived as pleasant with relatively mild trigeminal pungency.
3. Chemicals could not be used in their neat concentration but were diluted to match subjective intensity of an odour with ecological relevance to newborns, i.e., to Nutrilon in Pilot Study I — and then, to one selected concentration — to 1 000 ppm[4] solution of Vanillin in Pilot Study II. Two

[4] For simplification purposes and in order to easily express so very dilute concentrations of substances, we decided to use:
parts per million (ppm) abbreviation; i.e., one part of solute (our odorants) in one million parts of solvent (mineral oil or water) where one percent is equivalent to 10 000ppm.
parts per billion (ppb) abbreviation; i.e., one part of solute (our odorants) in one billion parts of solvent (mineral oil or water) where one percent is equivalent to 10 000 000ppb.

studies were needed to compared the stimuli's intensity to ecological valid stimulus to newborns, i.e., Nutrilon and then to a Vanillin used in Main Study. Vanillin was chosen because it appears to be hedonically pleasant for newborns (Bartocci et al., 2000).

1.1. Description of the eight selected odorants

In the following table, Table 1, there are eight selected odorants, each with their perceived hedonicity, trigeminal pungency, detection thresholds and recognition levels in adult population along with diluents chosen for this study (Burdock & Fenaroli, 2002; Leonardos, Kendall, & Barnard, 1969; Ruth, 1986; Saliba, Bullock, & Hardie, 2009).

Table 1: Eight selected Odorants in 4 categories according to their level of trigeminal pungency and perceived pleasantness

Odorants	Perceived pleasantness	Trigeminal pungency	Detection threshold	Recognition levels	Diluent
Trimethylamine	unpleasant	relatively strong	0.3-0.8ppb	0.2ppm	water
Butyric acid	unpleasant	relatively strong	240ppb-4.8ppm	9ppm	water
R-(–)-carvone	pleasant	relatively strong	2.7-600ppb	non-assessed	mineral oil
Eucalyptol	pleasant	relatively strong	1-64ppb	3.2ppm	mineral oil
Thioglycolic acid	unpleasant	relatively mild	non-assessed	non-assessed	water
Mercaptoethanol	unpleasant	relatively mild	120-630ppb	2ppm	water
Vanillin	pleasant	relatively mild	29ppb-1.6ppm	4ppm	water
Amyl acetate	pleasant	relatively mild	2-43ppb	37ppm	water

We realize that this is not the prescribed way to express the values of solutions. Neither does it reflect the way the solutions were prepared which was done by the chemists in laboratory of diversity and evolution of anaerobic protists, Charles University, Prague.

1. **Trimethylamine**

   A colourless, hygroscopic, and flammable tertiary amine has a strong "fishy" odour in low concentrations and an ammonia-like odour at higher concentrations. Trimethylamine is a product of decomposition of plants and animals, and it is used in food industry as an artificial flavouring for its meaty taste. Diluted in water, its 1% solution was presented to 5 to 12 year-old children in Soussignan & Schaal (1996).

2. **Butyric acid**

   A carboxylic acid with an unpleasant smell and acrid taste, with a sweetish aftertaste similar to ether. Butyric acid is found naturally in milk and butter and as a product of anaerobic fermentation. In food industry, it is often used to imitate milky taste. Diluted in water, its 0.125%, 0.031%, 0.0078% and 0.0039% solutions were presented to 3-day-old newborns in Soussignan et al. (1997).

3. **Carvone;** more specifically its R-**(–)**-carvone[5] enantiomers

   Carvone is found naturally in many essential oils in spearmint leaves and dill. R-carvone smells like spearmint leaves as opposed to its mirror image, S-(+)-carvone which smells like caraway seeds. For its specific flavour, it is often added to sweets as an artificial flavouring. Diluted in mineral oil, its solutions ranging from 10^{-6} to 10^{-3} were presented to 4-12 year-old children in Monnery-Patris et al. (2009).

4. **Eucalyptol**

   A naturally occurring colourless compound of a distinctive pleasant spicy aroma and taste. Eucalyptol is found in tea tree, camphor laurel, and bay leaves and its usage is ubiquitous in our culture. Diluted in mineral oil, its 50% solution was presented to 4-day-old newborns in Pihet et al. (1997).

[5] Previously known as L-carvone (L for Latin *laevo*) as opposed to D- carvone (D for *dextro*) now referring to as S-(+)-carvone.

5. **Thioglycolic acid**

   A colourless liquid with a strongly unpleasant odour, reminiscent of rotten eggs. In very low concentrations found in sugar beets, cane sugar, and unripe grapes. Diluted in water, its 0.5% solution was presented to 5- to 12 year-old children in Soussignan & Schaal (1996).

6. **Mercaptoethanol**

   A chemical compound that can act as a biological antioxidant. 2-mercaptoethanol has a rather unpleasant smell, weakly reminiscent of poultry. Diluted in water, its 1% solution was presented to 5- to 12 year-old children in Soussignan & Schaal (1996).

7. **Vanillin**

   An organic compound naturally occurring in vanilla beans. Synthetic Vanillin, especially its synthesised version, is as a flavouring agent in foods, beverages, and pharmaceuticals. Diluted in heated water, its 0.125%, 0.031%, 0.0078% and 0.0039% solutions were presented to 3-day-old newborns in Soussignan et al. (1997).

8. **Amyl acetate**

   An organic ester, the condensation product of acetic acid and 1-pentanol. With a scent similar to bananas and apples, Amyl acetate is used as a flavouring agent. Diluted in water, its 0.5% solution was used similarly in Soussignan & Schaal (1996).

As mentioned in the theoretical part of this thesis, the perceived hedonicity could be influenced by the intensity of the odorant. Therefore, two pilot studies were conducted in order to match these solutions in their perceived intensity.

1.2. Pilot study I

In Pilot Study I, stimuli of varying levels of dilution were matched on their subjectively perceived intensity compared to newborn's ecologically-relevant odour, i.e., to Nutrilon. This was because, as discussed previously, intensity of odours could influence its perceived quality (especially hedonicity) and thus affect the elicit hedonic responsiveness.

Therefore, the main aim of this pilot study was to compare the intensity of each dilution of the given odorant to the intensity of Nutrilon formula prepared according to instructions and find the one closest to it, which could be used as a measure of comparison for Pilot Study II. A secondary aim was to narrow down the range of concentrations of each odorant, thus fewer stimuli could be used in Pilot Study II.

For questionnaires, information sheets and informed consents regarding both of these pilot studies see attachment A.

1.2.1. Materials and Methods

Participants

Twenty participants (F=10) aged 24.6 ± 4.80 years were recruited in a building of Charles University of Prague. Inclusion criteria were: age 18-35 years, not having suffered from a head injury resulting in a coma, not a heavy smoker, and in a good respiratory health (participants were directly asked if they did not currently suffer from allergy attacks). If a self-admitted occasional smoker, the participant was asked not to smoke at least an hour preceding the testing. Each participant was informed on what taking part in this study would involve, and if they agreed, their contact information was collected and certain testing time was assign to them, according to their needs.

Stimuli

Because we could only assess the perceived intensity of stimuli on adult population, Nutrilon formula was chosen as most suitable as it is tailored to the neonates' not only nutritional, but also olfactory needs and thus, should represent an ecologically-relevant odour for a newborn. Nutrilon stimulus was fashioned following the directions on the powdered baby formula can. For each odorant a dilution range was established, see Table 2.

Table 2: The 45 stimuli used, their concentrations and codes. Each odorant was diluted in accordance with the previous studies and its molecular properties in either mineral oil or distilled water. Dilutions are given in ppm or ppb, where more appropriate.

Odorant (diluent)	Dilutions									
	10 000 ppm	1 000 ppm	100 ppm	10 ppm	1 ppm	100 ppb	10 ppb	1 ppb	0.1 ppb	0.01 ppb
Trimethylamine (water)			1	2	3	4	5	6	7	
Butyric acid (water)		8	9	10	11					
Carvone (mineral oil)		12	13	14	15					
Eucalyptol (mineral oil)		16	17	18	19	20	21	22		
Thioglycolic acid (water)			23	24	25	26	27	28	29	30
Mercaptoethanol (water)				31	32	33				
Vanillin (water heated to 60°C)	34	35	36	37	38					
Amyl acetate (water)	39	40	41	42	43	44	45			

These 45 stimuli were then divided into 2 sets in order for the participants not to experience fatigue during the session. Thus, two groups of participants were needed; one assessing the first set and the other the second one. Participants were divided into these two groups by chance as one set of stimuli was assessed on the first day

and the other on the second day. On the first day the set contained 23 stimuli, on the second only 22 stimuli.

Each day, the stimuli were prepared twice — first in the morning and then, once again, before the afternoon testing sessions — in order for them not to be older than 5 hours. For odorant presentation, sterile medical swab plastic sticks with cotton tip length 150 mm in polypropylene test tubes were used; same as in Main Study. Each time, the sterile cotton swab was dipped in the solution, one each, then carefully enclosed in its tube and the code of the solution was written on the plastic tube.

Questionnaire regarding the participants' olfactory abilities

To assess individual differences in olfaction, each participant was asked to complete a short questionnaire asking them to self-assess their olfactory abilities. Participants were asked to rate on a 5-point scale whether they thought they possess a better, or worse sense of smell than their peers and answer questions about their occupation and their living and work environment.

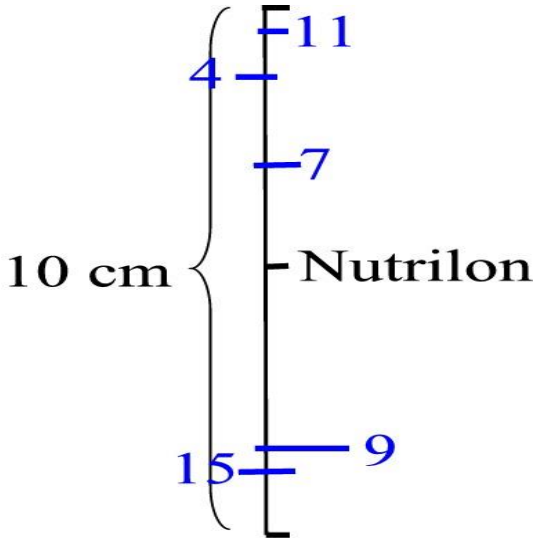
Furthermore, women were asked to state the date of their last menstruation and whether or not they were using hormonal contraception. Based on this data, no participant was excluded from the further analysis.

Visual analogue scale

A visual analogue scale (VAS), shown in Figure 1, was used to compare perceived intensity of the individual dilutions to that of the Nutrilon formula. It was 100 mm long with a mark in its centre labelled "Nutrilon". Participants were instructed to place a mark representing the given stimulus on the scale relative to the Nutrilon mark, with marks further from the bottom end of the scale indicating higher perceived intensity. Thus, stimuli that were perceived as less intense were marked in the 50 mm range below the Nutrilon mark, and similarly, the stimuli that were perceived as more intense were marked in the 50 mm range above the Nutrilon mark. As each stimulus was rated on a separate scale, resulting in 23, or 22 completed scales, depending on the day of testing.

Figure 1: Visual analogue scale (VAS)

On this VAS there are already some stimuli marked in a manner representing the testing procedure. In this example, stimulus number 7 would be perceived as more



intense than Nutrilon; number 9 as less intense.

Procedure

Data were collected on the 21st and 22nd of July, 2015. Testing sessions were scheduled throughout the whole day, each lasted for about 30 minutes. The session took place in a secluded, well-ventilated room. After the participants' arrival to the testing room, each was given a sheet with information regarding this study's aim with a short description of what their participation would entail. If still willing to take part, participants were asked to sign written informed consents and completed the short questionnaires to self-assess their sense of smell.

Participants were then instructed how to use the visual analogue scales and self-administer the stimuli, i.e. birhinally, about 1 cm from the nostrils. They were also asked to, if possible, disregard the perceived pleasantness of each stimulus and focus only on intensity ratings and if unable to perceive any odour note the number of the stimulus in the "I cannot smell" section. For each stimulus the following procedure was repeated; first, the participant sniffed the cotton swab with Nutrilon, then they sniffed the stimulus rated, and placed a mark on the VAS reflecting its perceived intensity relative to Nutrilon.

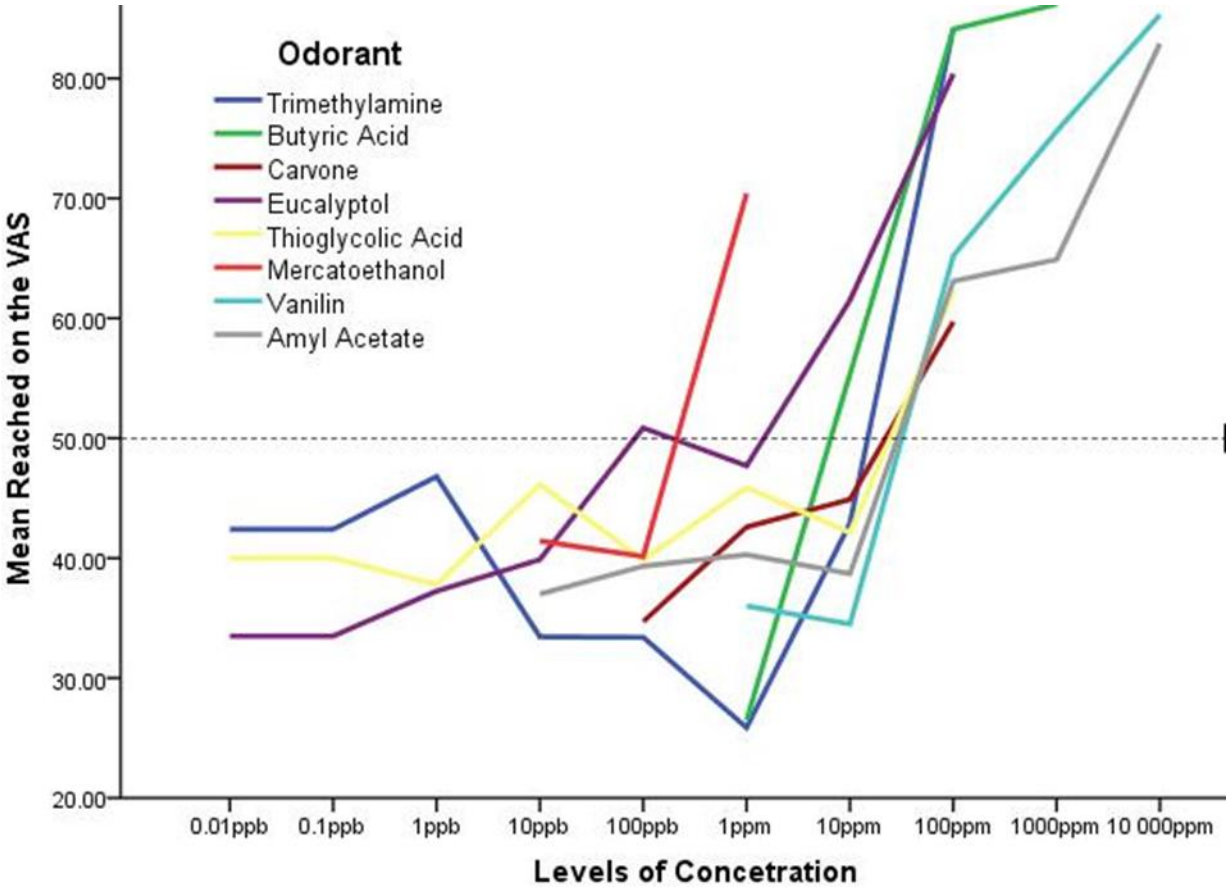
The samples with the solution were presented in a randomized order. The participants were encouraged to take breaks throughout the testing and have small

sips of still water. At the end of the session, each participant was offered to choose from a varying set of snacks as a token of appreciation for their time and effort.

1.2.2. Statistical Analysis

Data scoring reflected their position along the VAS measured from the bottom end in mm. For instance, a mark placed 70 mm from the bottom end of the VAS received a score of 70. Stimuli that the participants were unable to detect were disregarded from the analysis. Visual exploration of data revealed no outliers. Intensity ratings were averaged across participants disregarding their gender and visually compared to that of Nutrilon as seen in Figure 2.

Figure 2: mean VAS intensity ratings



1.2.3. Results

Based on the visual exploration, we chose a 1000 ppm solution of Vanillin (Soussignan et al., 1997) as the measure of comparison for Pilot Study II and 3 dilutions of each odorant with intensity ratings closest to that of Nutrilon. See Table 3 for the concentrations chosen, used as stimuli in Pilot Study II.

Table 3: The 21 stimuli selected for Pilot Study II, their concentrations, and codes.

Odorant (diluent)	Dilutions					
	10 000ppm	1 000ppm	100ppm	10ppm	1ppm	100ppb
Trimethylamine (water)				1	2	3
Butyric acid (water)		4	5	6		
Carvone (mineral oil)		7	8	9		
Eucalyptol (mineral oil)		10	11	12		
Thioglycolic acid (water)				13	14	15
Mercaptoethanol (water)				16	17	18
Amyl acetate (water)	19	20	21			

1.3. Pilot Study II

This pilot study was building on the results of Pilot Study I. While using analogical design, our aim here was to match the intensity of the pre-selected stimuli to that of 1000 ppm solution of Vanillin and thus, choose just one concentration of each odorant for Main Study. As the design of both pilot studies was kept as similar as possible, only the differences are stated in the following paragraphs.

Twenty participants (F=10) aged 28.9 ± 2.61 were recruited, this time in the building of National Institute of Mental Health. The participants of this study were older, as the sample consisted mostly of postgraduate students and employees of this research centre, no undergraduate students were recruited.

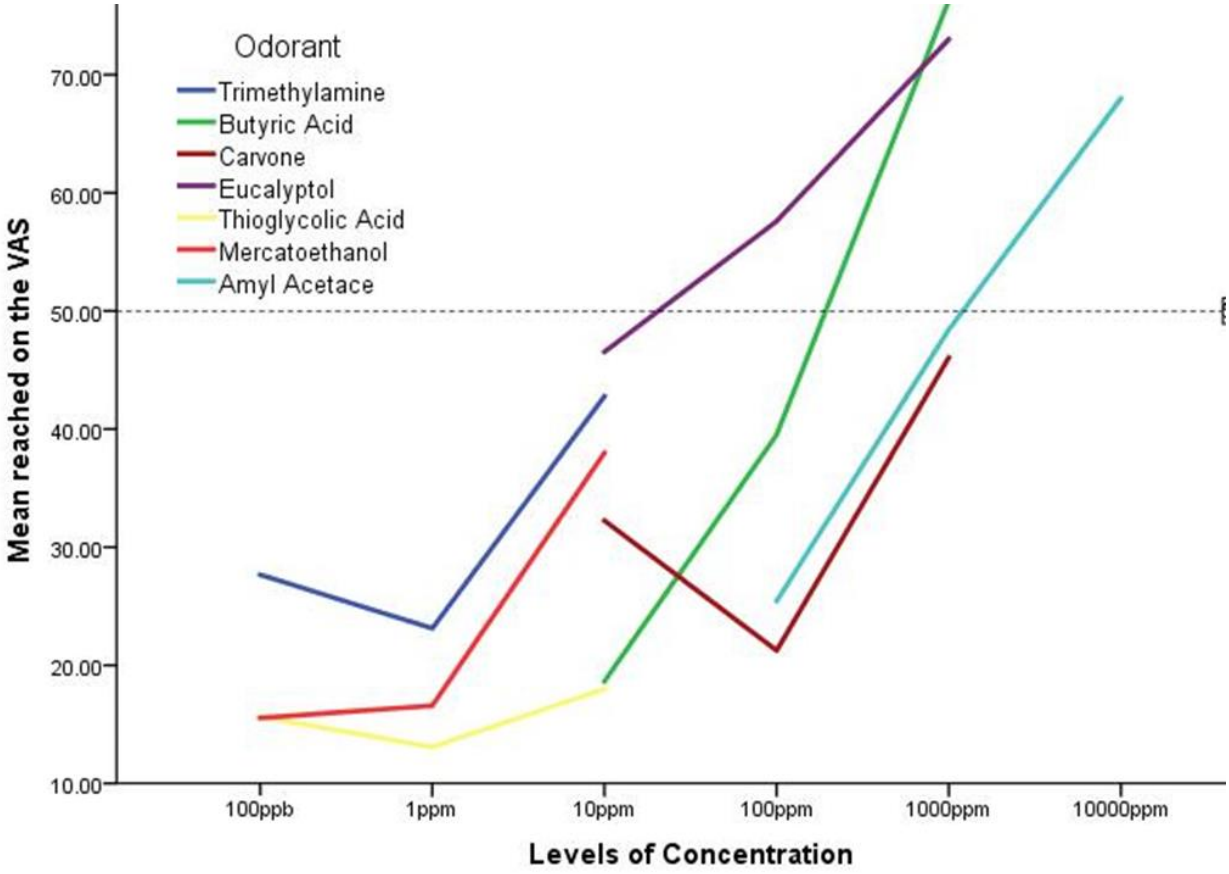
Stimuli employed were the 3 solutions of each odorant given in Table 3. The preparation of stimuli was kept exactly the same as in Pilot Study I. However, here we did not divide the stimuli into two sets; the whole set of 21 stimuli was presented to each participant.

Testing session yet again lasted no more than 30 min and took place on the 4th and 5th of August, 2015. The same VAS were used, only with the 1000 ppm solution of Vanillin in the middle instead of the Nutrilon. Data was processed and analysed analogically.

1.3.1. Results

For results see Figure 3.

Figure 3: Mean VAS intensity ratings



1.4. Implication for Main Study

As stated before, both of these pilot studies were performed in order to ensure the best selection of odorant concentrations for Main Study. After visual exploration of the results of both pilot studies, we finally decided on the following levels of concentration of odorants. Concentration of selected stimuli for Main Study are shown in Table 4.

Table 4: selection of odorant concentration

	Relatively strong trigeminal pungency		Relatively weak trigeminal pungency	
Concentration	Unpleasant	Pleasant	Unpleasant	Pleasant
10ppm	Trimethylamine (water)		Mercaptoethanol (water)	
100ppm	Butyric acid (water)	Eucalyptol (mineral oil)	Thioglycolic acid (water)	
1000ppm		Carvone (mineral oil)		Amyl acetate (water) Vanillin (water heated to 60° C)

2. MAIN STUDY

In newborns — contrary to adult population — no, or at least very limited, previous exposure to the odours might be expected; an experience that could and would influence the quality perception of an odour and thus interact with the response. The antenatal experience with odorants, though possibly significant, was not taken into account as it could not have been controlled for. As discussed before, full-term healthy newborns already possess a fully functioning olfactory system. Therefore, their perception of odorants should, more or less, mimic the one of an adult population with occurrence of autonomic measures as discussed in the theoretical section of this thesis These depend on the behavioural state of the person in question (Sullivan & Toubas,1998; Soussignan et al.,1997), contextual cues (Oberfeld et al., 2009) and the nature of the odour itself, its physicochemical and perceptual properties. Of these, of greatest relevance to the present study were intensity and pleasantness. Intensity of all the stimuli employed was manipulated to match that of an odour ecologically relevant to neonates. Pleasantness was handled so that groups of odours with a strong/weak trigeminal component were further subdivided according to hedonic ratings reported in adult literature. Hence, the effect of perceived pleasantness could be disregarded in the analyses reported in this thesis. This was because the two groups of stimuli carrying a relatively strong vs. weak trigeminal component contained two pleasant and two unpleasant stimuli each.

Not only the odorants presented could have influenced the newborns responses, their behavioural state has also the potential to alter their reactions. Newborns in the quiet awake state respond to a familiar odour with an increased behavioural activity, while crying infants will stop crying (Sullivan & Toubas, 1998). Moreover, trigeminal impulses have a greater effect on the cardiac and respiratory response during sleep (Heiser et al., 2014). Therefore, we chose the state of irregular sleep which best represents the REM phase of sleep in adults, non-existent in newborns (Prechtl, 1974). Besides, the level of the newborns satiety is also important and could influence their responsiveness, especially their orienting responses to biologically relevant signals (Soussignan, Schaal, & Marlier, 1999).

Yet another factor that needed to be taken into consideration was the mode of odorant presentation. In our study, we chose the birhinal mode of presentation as monorhinal presentation was shown to be affected not only by the odorant's potential to stimulate the trigeminal nerve, a fact that is discussed in the third chapter of this thesis, but also by the right or left handedness of the individual. Even more interestingly, it appears to be influenced by lateralization of the brain's hemispheres, with the left one being the more predominant one and thus more capable to discriminate an odorant (Doty & Kerr, 2005).

Last, but not least, newborns' method of feeding has to be accounted for as it determines, to a certain degree, their psychophysiological and behavioural reactivity; breast-fed newborns appear to be more irritable and less consolable then their bottle-fed peers, however, they seem to be better physiologically organized (di Pietro, Larson, & Porges, 1987).

2.1. Aims

The purpose of this study is to test whether unfamiliar odorants with contrasted trigeminal intensity (relatively strong vs. relatively weak) elicit differential autonomic response, operationalized in terms of heart rate variations, in newborns. Previous literature shows that heart rate correlates with perceived pleasantness in adults, and is thus treated as indicative of arousal magnitude and, in the final analysis, of odour hedonics. Taking into account the existing evidence, we hypothesise that there would be a difference in the heart rate frequency, in newborns upon their presentation with unfamiliar odorants possessing contrasting levels of trigeminal component.

2.2. Materials and Methods

Participants

Participants' Inclusion and Exclusion Criteria

For the purpose of our study, we have set the following sample criteria that eliminated the number of births potentially eligible to us by 60 % (the hospital's

average is 13 births per day). Thus, at times, we had to make some exception, as mentioned below, in order not to reduce our potential sample even further.

Delivery's Criteria

As regards the method of the delivery, we excluded births that required the use of forceps and/or vacuum extractors, general anaesthesia, other than the typical vertex (head-first presentation) delivery, and C-section. Variables that were not part of exclusion criteria but were monitored in the questionnaire were: the exact positioning of the infant's head while appearing through the vaginal canal, the length of labour (from the 1st felt contraction till the delivery of the newborn), the use of epidural anaesthesia or any other pain management (i.e., the use of Entonox, a quickly metabolised mixture of nitrous oxide and oxygen is that is sometimes used during the delivery to relieve the pain), episiotomy or natural tearing of the perineum and/or the posterior vaginal wall, any induction or acceleration medication, and gestational hypertension.

We included births where oxytocin was administered intravenously to the mother. In the Czech Republic, its application is commonly prescribed in the third stage of birth unless the mother specifically disagrees, signing a form stating so. As far as we know, no data about the effects of artificial oxytocin on neonatal responsivity exist. However, we excluded births where it was given to the mother earlier than in the third stage of birth, that is, with the baby still inside the mother and thus directly influenced by it.

Mother's Criteria

Only the mothers who were younger than 40 years of age were included in the study. Other, potentially confounding variables included age, nationality, marital status and socioeconomic status of the mother's family, smoking during pregnancy, dietary restrictions and preferences, their general health, and how they felt during the pregnancy. Also their levels of stress as experienced throughout the last moth preceding the delivery was assessed using the translated version of Perceived Stress Scale (PSS; Cohen et al. 1983).

Any over the counter medication was checked for. We excluded mothers who were taking any prescription medication other than Thyroxine[6] substitution as the prevalence of thyropathy is high in the Czech Republic. The T4 substitution, mostly in form of Euthyrox, was checked for along with its exact dosage. Also we checked for the use of alcohol and illegal substances.

Newborn's Criteria

Exclusion criteria for the newborns were, firstly, gestational age under 37 or over 42 weeks, which is one of the requirements to meet the physiological birth definition. Secondly, they included height and weight percentile outside the norm, that is, below the 5th or above the 95th percentile. Thirdly, newborns with any of the three Apgar scores[7] equal or lower than 7 were excluded, as were those with infant jaundice serious enough that it would require phototherapy.

Other than that, in the days before the presentation, we monitored for the infants' weight gain, their feeding patterns (how much and how often they were fed), whether or not they were breastfed or whether they were on some form of feeding substitution. The newborns' age was set as two or three days old, mostly for the logistical reasons, as we needed to get their mother's approval on the preceding day and we were not allowed to disturb her on the day of the delivery (i.e., the first day). The upper limit was set so as to reflect the fact that the hospital stays of healthy mothers and newborns in the Czech Republic is usually four-day long, with them being discharged early in the afternoon.

[6] Thyroxine, or T4, along with its active form triiodothyronine (T3) are hormones produced by the thyroid gland that are primarily responsible for regulation of metabolism. In hypothyroidism, where thyroid gland is producing not sufficient amounts of one of these two hormones, their substitution in forms of oral medication is needed (Formenti et al., 2016). In the Czech Republic, only T4 substitution are administered, in other countries both hormones are often substituted, if need be.
[7] A measure of the physical condition of a newborn infant in 5 domains: breathing effort, heart rate, muscle tone, reflexes, and skin colour. Each domain is scored with 0, 1, or 2, depending on the observed condition. In the Czech Republic, each newborns is assessed in the first, fifth, and tenth minute after their delivery, amounting in the three scores.

Participants' Recruitment

In accordance with the requirements of the hospital ethical committee, the mothers had to be first approached by the attending physician, shortly explaining the aims of our study to them. Only after the mother gave her oral consent, could the physician tell us her name and give us information as to where to find and contact her. Once informed, mothers provided their cell phone number to secure future contact.

Preliminary analyses of approximately (39 % of all cases) of data have shown that more than two thirds of our successfully recorded newborns were male. Thus, for the remainder of the study, we were focusing on the girls; eligible mothers of baby girls were approached and recorded first.

The Reasons Behind High Participants' Dropout Rates

Dropout reasons were monitored throughout the study. Physicians reported that circa 60-70 mothers have refused to provide oral consent, while 168 mothers (F=95) gave permission to be approached by us. Out of those, 128 mothers (F=72) agreed to participate in our study, signed informed consents, and completed the questionnaires. However, only 50 newborns (24 F) have been successfully recorded in the end. The dropout reasons have been categorized based on the participants' general refusal to participate or dropout just before or after the testing had already started. Another factor was internal versus external reasons, internal meaning those related to mother and child's physical and psychological state, external those relating to our technical and logistical capability to execute the testing session successfully.

1. **Refusal to participate.**
   Here, we included only those mothers we had the opportunity to inform about the study aims ourselves. Though we did not inquire the reason behind their refusal to participate, some mothers willingly shared that they were afraid our study could harm their newborn; allergic reaction to unfamiliar odorants was often mentioned.
2. **No presentation, external reasons.**
   They were days when we were unable to even start with the presentation, e.g., doctors were unable to spar time in their tight schedules or mothers

were released earlier because beds were needed due to a department being closed for renovation.

3. **No presentation, internal reasons.**
   Here, the main reason was newborn fallen ill, or did not fall asleep during the time allotted to us by the hospital or its mother changed her mind about the participation.
4. **Presentation failure, external reasons.**
   Throughout the study we faced several technology difficulties, e.g., the recording camera had to be changed twice, medical equipment was not working properly.
5. **Presentation failure, internal reasons.**
   The sole reason here was the newborn's waking up during the presentation with which the recording was stopped and the newborn had to be disregarded from our study.
6. **Presentation success.**
   Only these newborns entered the following analysis and are discussed in the Participants sample.

All categories with their absolute and relative frequencies are shown in Figures 4 and 5 for boys (N = 73) and girls (N = 119), respectively.

Figure 4: Absolute and relative frequencies of the six main reasons behind dropout in boys

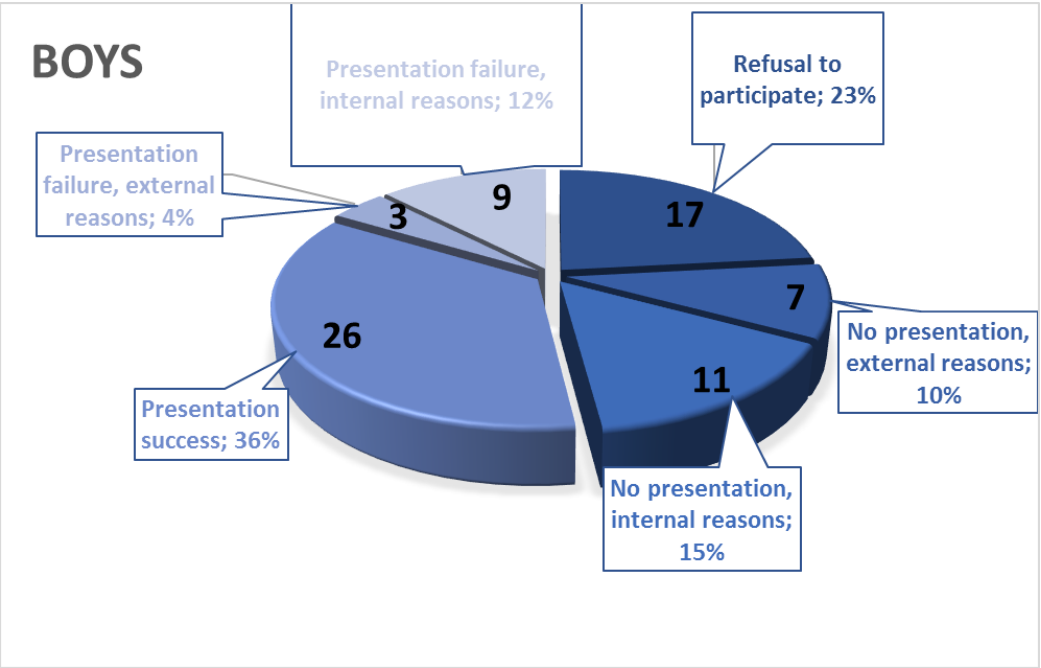
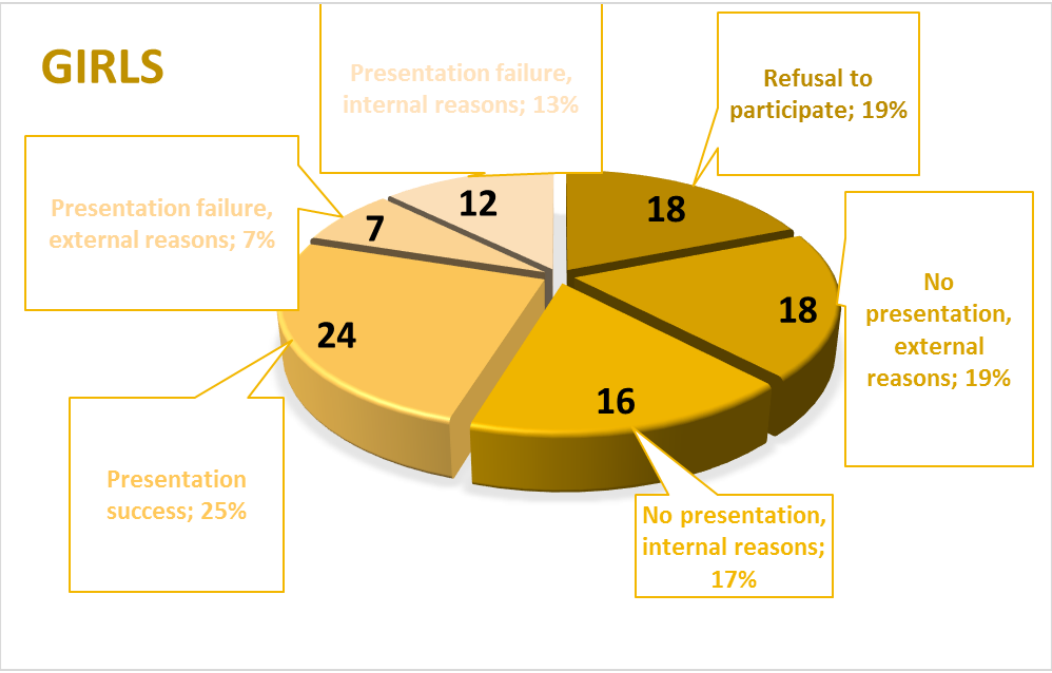


Figure 5: Absolute and relative frequencies of the six main reasons behind dropout in girls



Description of the Participants' Sample

Mothers:

- age 32.08 ± 4.07 years (range), 28 were primiparous;
- 46 were Czech, 30 had BA degree or higher, and reported to have family income of 20-50 000 CZK;
- 30 were married, 17 single, and 3 divorced;
- 6 were vegetarians, 4 were smokers, 21 reported occasionally drinking while pregnant and one smoking marihuana;
- 9 were using general analgesics, 7 were using T4 substitutes.

Newborns:

- at the moment of testing the newborns had an average age of 53.18 ± 8.83 h;
- length of their birth was 325.18 ± 180.28 min, all but one appeared with their face towards the mother's rectum (occipito-anterior position), their mean Apgar score was 9.67 ± 0.71;
- 42 newborns were breastfed (out of which 8 received additional servings of Nutrilon), 3 were on Nutrilon, and 5 were requiring no food yet.
- their temperature assessed anally was 36.85 ± 0.25°C.

Questionnaires

All questionnaires were in Czech and are enclosed in Attachment B along with information sheets and consent forms used in this study.

General questionnaire

Each mother was asked to complete the general questionnaire (see attachment). Its purpose was to check for the possible confounding variables described in the participants' sample. It was divided into 4 main parts: with questions relating to the mother demographics range, e.g. "What is the highest level of education you have completed?", to her diet, e.g., "How often do you eat spicy foods?", and to her general well- being, e.g., "Did you experience some ailments throughout your pregnancy?". The last part was shown to the mother asking her to agree a doctor would look into her medical chart and filled those question for her, to which all mothers gave their oral consents. The doctors were filling in information about the delivery (e.g., use of forceps or vacuum extractor, episiotomy or natural tearing of the perineum and/or the posterior vaginal wall, form of anaesthesia and pain management, the length of labour) and newborn (e.g., perinatal hypoxia, Apgar score, weight and length)

Evaluation of the odorants perceived quality

Every mother was also presented with the two odorant samples that would be used with her newborns in the following day. She was asked, without being told what the odorant was, to describe the odour using 16 characteristics provided (e.g., burning, cooling, bitter, sweet ect.), on a seven-point scale rate its perceived pungency, familiarity, pleasantness, and intensity and whether she felt it was cooling. Last question was open, asking the mother to write of what, if anything, the smell reminded her. This questionnaire was used mainly to introduce mothers to the odorants their newborns would be presented to the next day.

Perceived Stress Scale

To obtain self-reports of the levels of stress the mothers experienced, a Czech version of the freely accessible Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983) was used. Though originally designed for use in community samples with at least a junior high school education, the questions are of a general nature. Because of its shortness and generalizability, we employed it in our study.

It contains 10 questions asking about the participants' feelings and thoughts during the last month. Six questions relate to negative emotional experiences, e.g., "In the last month, how often have you been upset because of something that happened unexpectedly?", and 4 relate to positive experiences, e.g., „In the last month, how often have you felt confident about your ability handle your personal problems?". A 5-category frequency response format is used ("never", "almost never", "sometimes", "fairly often", "very often"). The responses are scored 0, 1, 2, 3, or 4, with items relating to positive experiences reversed first, and a total score is computed. For a Caucasian woman in her thirties the norm is 16.78 ± 6.86 (Cohen & Janicki-Deverts, 2012). The mothers from our sample scored slightly higher (18.98 ± 7.24) as well expected given they were asked to assess the level of stress a moth before delivery.

Data collected from the newborn

Data was collected using a medical 3-lead electrocardiogram (CareScape B450) and pulse oximeter (SpO2 Masimo LNCS YI), both borrowed from the hospital. A tent (1x1x1.7m) was set over the newborn, in order to standardize the condition of each session. The tent was fitted with the camera (GoPro HERO+) and dimply lit and its lining protected the newborn not only from other lights in the room, but also from the random air circulation.

Each newborn's temperature was taken anally, right after the testing session was over, to ensure the newborn was not having a high fever. No newborns did. Moreover, to assess the newborn's autonomic responsiveness, the ECG was used. With it, we recorded two curves. One contained the rate and rhythm of his/her heartbeats which were further analysed in this thesis. The other measured the

newborn's electrical impedance between the two electrodes located in the right and left subclavicular region; a curve that will enable us to assess the newborn's breathing frequency which is a task far outreaching this thesis aim.

The same can be said about the data collected from the pulse oximeter, which, placed on the newborns palm, was used to measure its levels of saturations throughout the testing. In the future we hope to use them not only to measure the newborns' oxygen levels, but also, together with the data from the electrical impedance curve, calculate the depth of the newborn's breath.

On the behavioural level, a digital camera was used to record new-borns' oral, facial, and head movements. These data are not part of this thesis and will be used later to assess the visually expressed reactivity of a newborn to each presented odorant.

Procedure

To respect the hospital daily schedule, we came up with this following mode of testing. The recording took part in the afternoon of the third or fourth day after the delivery was set. The third day was preferred because, as mentioned above, some mothers were released a day earlier.

The afternoon of the preceding days — i.e., Mondays, Tuesday, and Thursdays — were dedicated to informing the mothers and often times the fathers as well about the general aims of our study, them signing informed consents —as one of the requirements of the ethics committee was, as mentioned earlier, for the mothers to be informed at least a day before the actual odorants' presentation. The afternoons were preferred because they are the times of visiting hours and mothers are free to do as they please with no medical visit and/or examinations. Information sheets and consents are enclosed in the Attachment section. For these reason, the procedure was spread across two days.

Day One

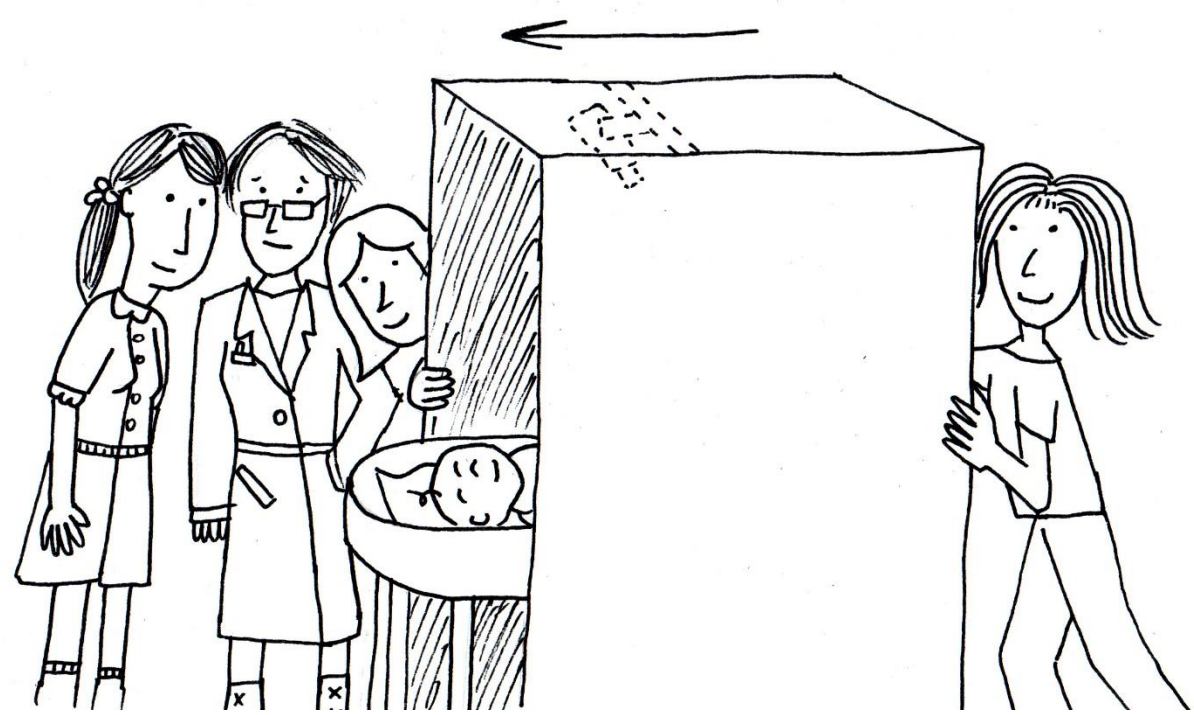
In the morning, the cooperating doctors looked into medical charts of all women who gave birth and were located in the physiology department of the hospital. Out of those, they selected the mothers fitting our criteria and visited them, informing them briefly about our study, and asking whether or not the mothers were interested in us coming later in the afternoon and explaining to them what would the participations entail. If they showed interest, their names along with their hospital room numbers and bed were given to us.

In the afternoon, we came to the hospital. After careful explanation about the general aim and design of the study, the mothers were given a leaflet summarizing all information they need, along with contacts on the authorities responsible for this study. If they wanted to take part in this study, we gave them informed consents to read and sign. If they wished so, we gave them few hours to think it over, returning later in the afternoon. Either way, the mothers were assured that their cooperation was voluntary and they could end it anytime they pleased. With all that in mind, they were asked to sniff and using the olfactory questionnaire, asses the two odorants that would be presented to their newborns during the following day's testing session. The odorants were presented on the sterile cotton wool swabs, one drop of the liquid on

each swab. The same presentation was used with the children with the exception of the mothers being allowed to sniff the odour as long as they like. After that, the mothers were told the exact odorant their children would be exposed to during the testing with the added information where and for what purposes the odorant is being used in the food industry. Furthermore, mothers were asked to fill out the questionnaires about their socio-economic status, general health, and well-being.

Before our leaving, the mothers received contact information where they could text or mail to should they change their minds regarding their participation, were told when exactly on the following day the presentation would occur, and were asked to, if possible, feed their children an hour or so before this. As one of our conditions was that no testing should take place earlier than a half an hour after last feed. Moreover, by following this procedure, we were trying to ensure the infants' behavioural state (irregular sleep).

Day Two



On the following day, we came to the hospital a half an hour earlier before the first planned testing to set out the room for the presenting session. A tent was put out. The heart and oximeter monitors were set, along with the computer that was recording all the data. Before each session, the temperature and humidity of the room

was measured and, if possible, standardized. Thus the temperature across all presentation was 24.78 ± 1.68°C with humidity 39.04 ± 5.45%.

Once everything was ready, we visited the mother who was first on our lists and asked her whether she still agreed with the participation, when the last feed was, and checked if the baby was sleeping. If everything was to our standards, we transferred the newborn in his cot to our testing room and asked the mother to accompany us, should she wish to do so. Most mother agreed to and were thus present for the whole testing session; generally, a calming effect of mother's presence on a newborn could be expected (Nishitani, et al., 2009). The mother witnessing the presentation were asked to, if possible, not touch and/or talk to their newborn while the session lasted. No mother was prevented from calming her newborn should she wished to do so. If some of our standards were no met, e.g., the newborn was awake, we asked the mother to kindly let us know once it did fall asleep or to try and feed it and contacted her in another hour or so. If other mothers were waiting the day to let their newborns be tested, we moved to them. If not, we waited.

Back in the room, the baby was stripped of its clothes in order to attach the electrodes of the 3-lead electrocardiogram; one in each of the subclavicular area and one in the upper left side of the abdomen. Thus, we tried to eliminate any interference of the electrical activity recordings coming from the movements of the newborn's limbs. When the electrodes were attached the newborn was yet again dressed and safely tucked in the hospital cot, which was set to be parallel to the floor. Normally, the newborn's reclining cot is set in 45-degree angle, in order for its head to be slightly elevated and thus preventing regurgitation of the semi-indigested food. We opted for the parallel-to-floor position in order to ensure the chances of the infant to be in the irregular sleep (Prechtl, 1974) and not soundly asleep. It should be noted here that the doctor was present to each session, attaching all the medical equipment to the newborn's body, undressing and dressing it, and assessing the behavioural state of each newborn before and during the presentation. If the newborn remained asleep, the doctor placed the pulse oximeter on its right hand palm and then the newborn was left for few minutes lying in the cot in the tent undisturbed.

In the meantime, the odorants' stimuli were prepared by the other researcher, again one drop of the odorant on each sterile cotton swab, then each swab was placed back into its plastic tube which was marked according to the order the odorant should be presented during each session unique to each participant. This process allowed for the presenting researcher being blind to the nature and order of the stimuli presented. This being done and with the newborn still asleep, we started recording. Each infant was recorded on the behavioural level by the digital camera set in the roof of the tent, recording oral and facial movements of each newborn and on the autonomic level by the 3-lead electrocardiogram and pulse oximeter.

Presenting Design

The whole recording session lasted for 8 minutes, many aspects of which were, yet again, adopted from the methodology used in Soussignan et al. (1997), and was divided into these following sections.

First we recorded 80 s of baseline measurements for each newborn, in which the newborn was left undisturbed lying in the cot, placed in the tent. This baseline measurement was followed by 3 consecutive trials — one for each stimulus — each of trial consisted of:

- **10 s interval,** a visual and mechanical mark was created in the recording to mark each presented odorant.
- **10 s interval.** the odorant was placed approximately one centimetre under the nose of the sleeping newborn (birhinal-orthonasal presentation). If the newborn moved his/her head in one direction or other, the odorant remained in the original position, allowing the newborn the possibly avoidant move.
- **70 s interval,** after the stimulation, the baby was yet again left sleeping undisturbed in the tent while all his/her behavioural and autonomic reaction were recorded.

For the whole trial procedure see Figure 6.

Figure 6: Representing the procedure of one trial

After the session ended, all electrodes were taken off the newborn's body. Either way, the newborn was returned to its mother lying in the cot. The mother asked to take the newborn's temperature anally, a process that the mothers are well accustomed to, as they do so at least two times a day. In the meantime, we photographed her breastfeeding chart using a cell and gave the doctors her questionnaire to fill the information needed from the mother's medical chart.

The testing ended with us offering the mother a 300 CZK gift certificate as a token of appreciation for her child partaking in our study and asked whether she was interested in the video recording of her baby's performance. If she were, her mail address was noted and later the video was shared with her via a safe online service.

2.3. Statistical Analysis

All data was digitized using Excel; the data from the 3-lead EKG were downloaded and stored and with the help of a PhD student from Czech Technical University in Prague, transformed to an Excel appropriate matrix using MATLAB software. After uploading into Excel, the heart rates of each newborn were divided into the intervals reflecting the study design. First 80 s were used to determine a heart rate baseline for each child. The rest was divided into eight 10 s intervals, with the second one corresponding to the time when the odour was presented. Data across each of these intervals have been averaged to obtain 8 repeated measures plus the baseline measurement. All further analyses were carried out with SPSS v. 22.

We ran 9 general linear models (GLM) with different main factors and interactions added to the model comparing their reached partial eta squared values to estimate the effect sizes and statistical power of these models as these estimates

could be informative in addition to p-values (Levine & Hullett, 2002). Information about these models is enclosed in Attachment C.

Based on these comparisons, a GLM was selected for the final analysis of all the three trials with continuous heart rate measurement averaged into 8 repeated measures over 10 seconds each as within-subject variables, odorant (mild/strong trigeminal stimulant) and newborn sex as between-subject factors, and the baseline heart rate (averaged across the initial 80 s) as a covariate. A simple-first contrast was used for within-subject effects. Same model was used for all the three trials. We did not include the preceding stimuli presented into the model as no influence of the preceding stimuli presented was found in the second and third trial. On the other hand, the baseline has proven to be influential, as it strongly correlated with the heart rate means across all trials.

2.4. Results

Descriptive Statistics

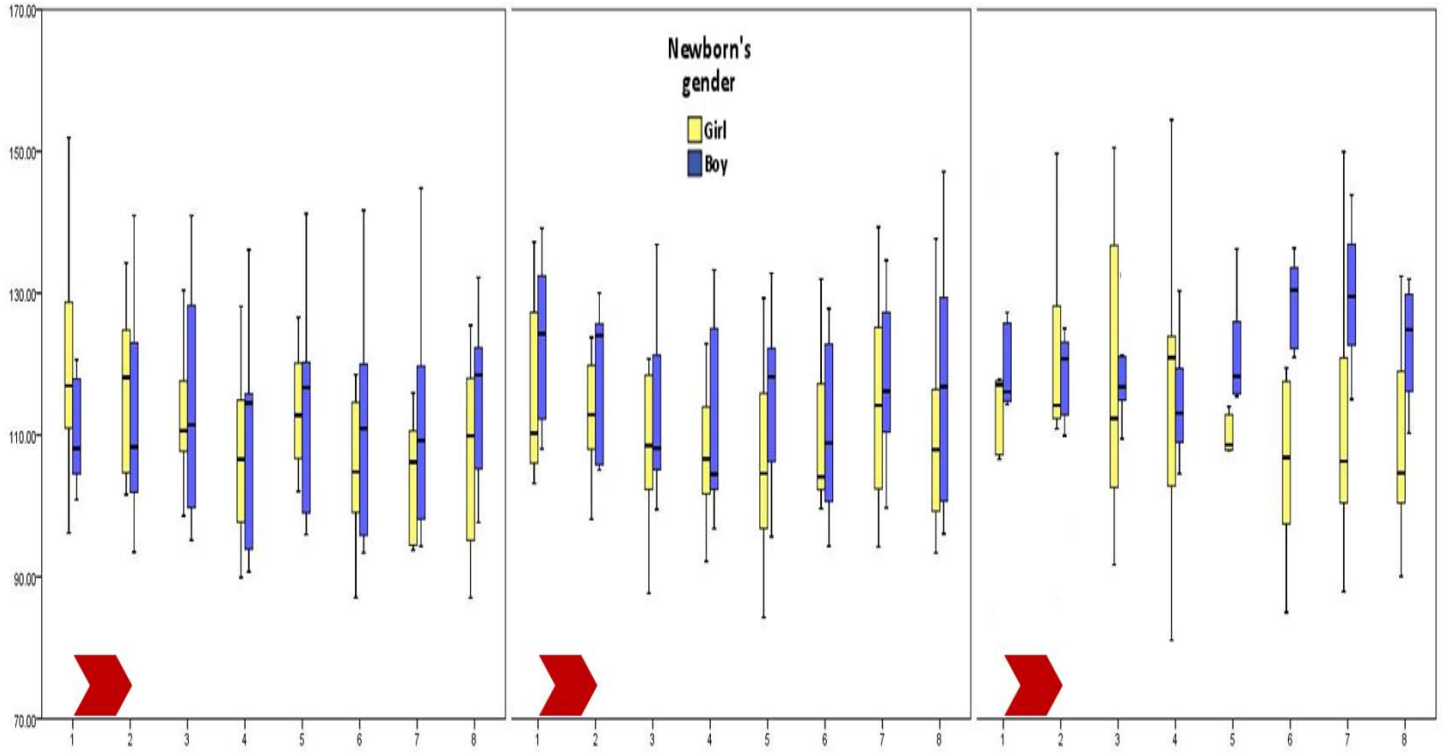
The descriptive analysis of the data was performed first to check for potential outliers using visual exploration of histograms and boxplots. No outliers were found. There was one participant with constantly higher levels of heart rate per minute. However, given the difficulty these data were obtained we decided to include him in the following inferential analysis. Data normality was checked by visually examining histograms and P-P plots of all relevant variables and Shapiro-Wilk's W tests, none of which were reaching statistical significance. Therefore, parametric tests were used.

The description of heart rate variation throughout the whole presentation

In Figures 7-9, the averaged heart rate values (repeated measures) across the eight 10 s intervals are given for each of the 3 consecutive trials. Specifically, heart rate variation across repeated measures in blanks, stimuli with a relatively mild and a relatively strong trigeminal component, respectively, is shown.

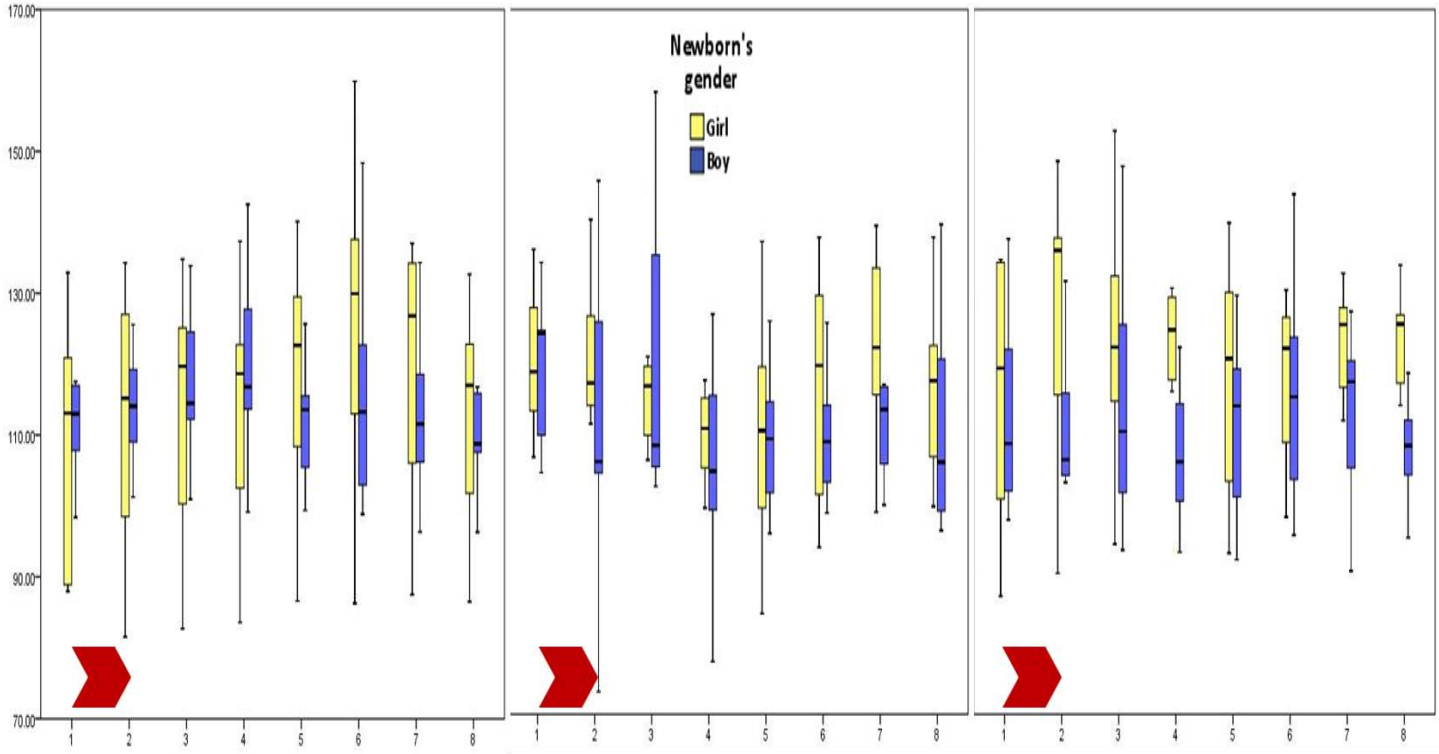
Figure 7: Boxplots for heart rate means in 3 trials for the blank stimuli.

On the X axis are the 3 consecutive trials — each consisting of 8 heart rate means — marked by the red arrows are the three time periods in which the newborns were presented with the blank stimuli. On the Y axis is the heart rate, ranging from 70 to 170 heart beats per minute.



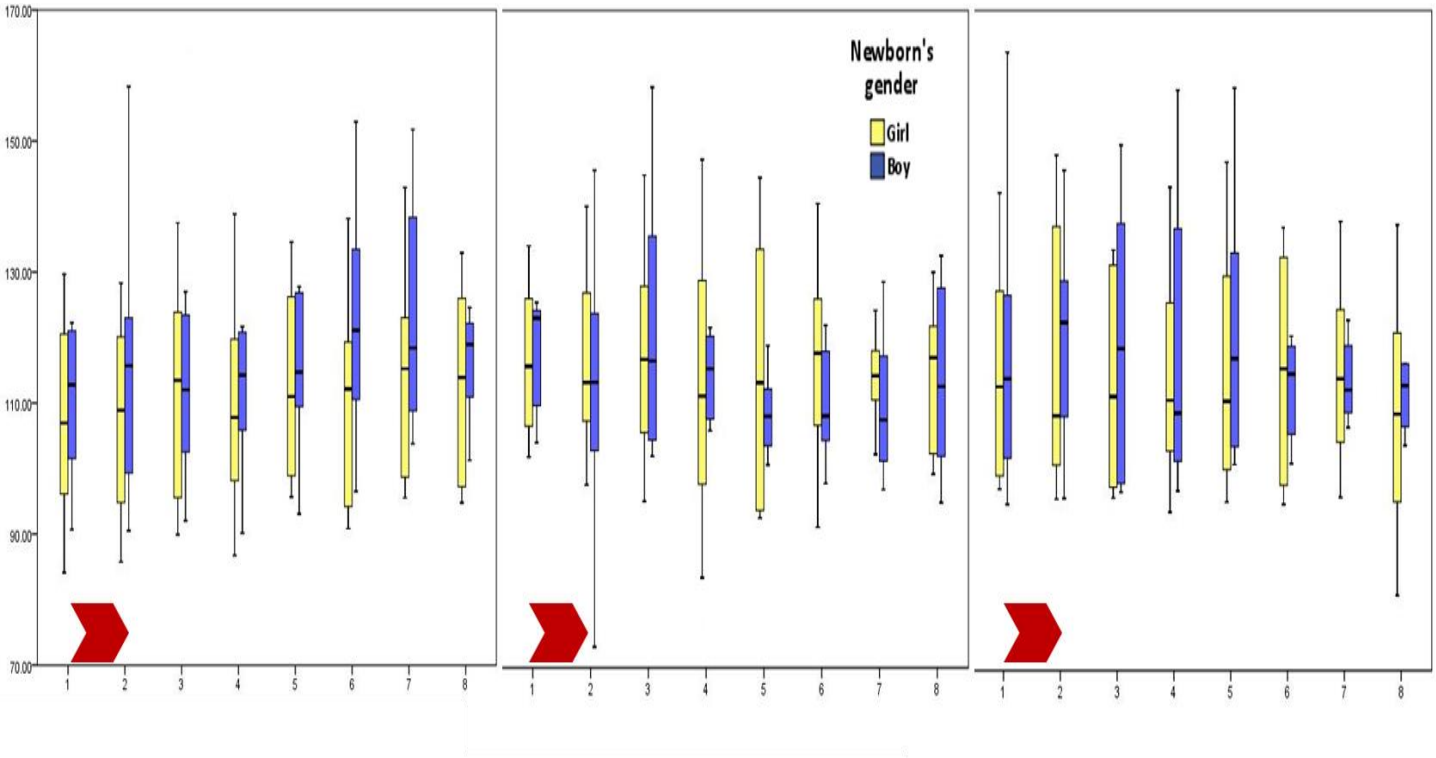
Graph 8: Boxplots for heart rate means in 3 trials for the stimuli with a relatively mild trigeminal component.

On the X axis are the 3 consecutive trials — each consisting of 8 heart rate means — marked by the red arrows are the three time periods, in which the newborns were presented with the stimuli with relatively mild trigeminal pungency. On the Y axis is the heart rate, ranging from 70 to 170 heart beats per minute.



Graph 9: Boxplots for heart rate means in 3 trials for the stimuli stimulating trigeminal nerve more.

On the axis are the 3 consecutive trials — each consisting of 8 heart rate means — marked by the red arrows are the three time periods in which the newborns were presented with the stimuli with the relatively strong trigeminal pungency. On the Y axis is the heart rate, ranging from 70 to 170 heart beats per minute.



Inferential Statistics

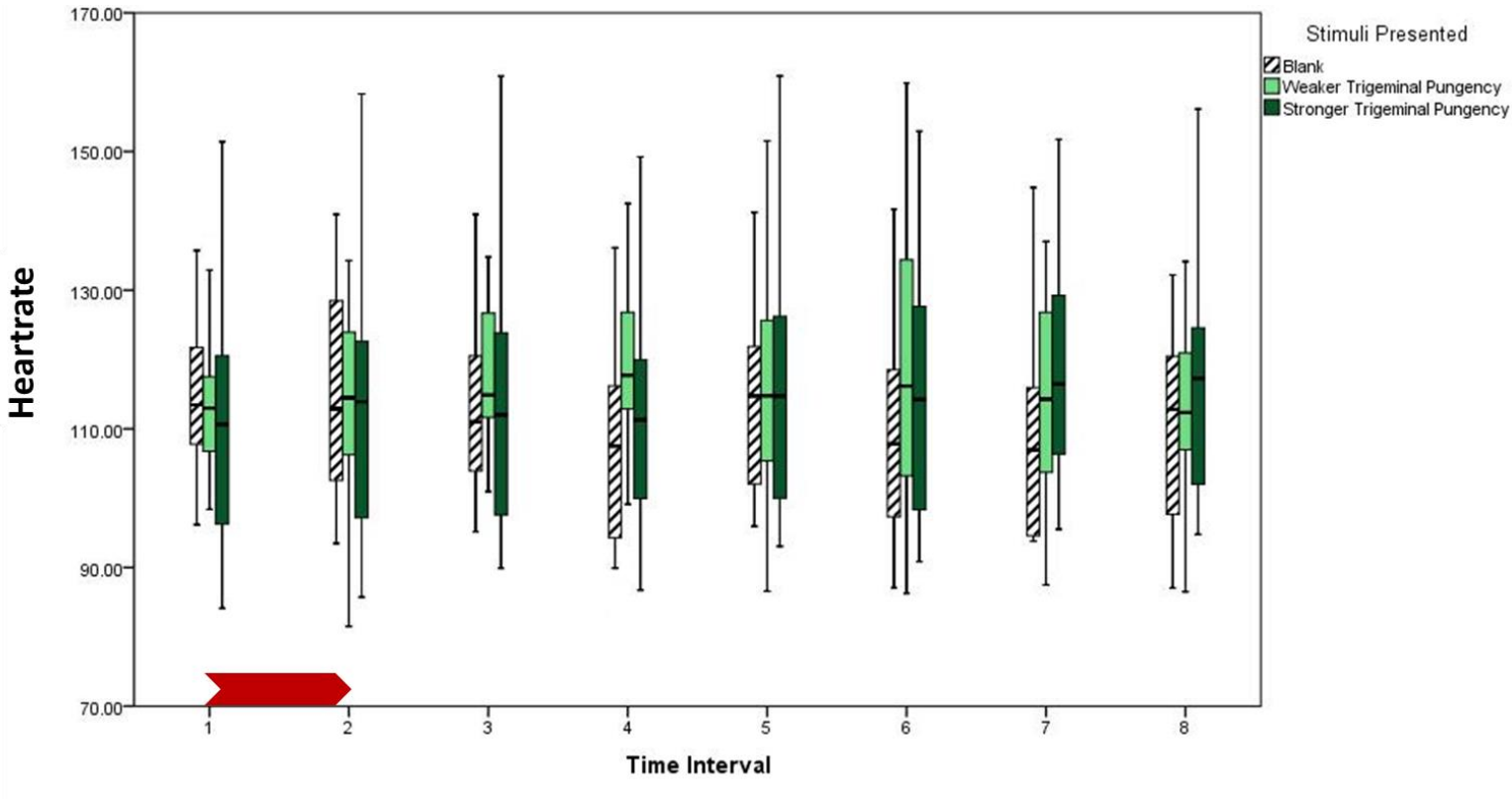
First Trial

Multivariate tests showed a significant effect of the within-subject variable, Pillai's Trace = .412, $F(6,38) = 4.445$, $p < .01$, its significant interaction with baseline heart rate, Pillai's Trace = .405, $F(6,38) = 4.306$, $p < .01$, as well as with the odorant presented, Pillai's Trace = .546, $F(6,38) = 2.439$, $p < .01$.

Hence, tests of within-subject effects on of the first trial revealed that newborns' heart rate exhibited variation across repeated measures, $F(6,38) = 4.445$, $p < .01$, and that there was an interaction of heart rate variation and the baseline heart rate measurement, $F(6,38) = 4.306$, $p < .01$. Most importantly, there was an interaction of the presented odorant with the course of heart rate variation, $F(12,78) = 2.439$, $p < .01$. No significant interaction was observed between the heart rate variation and the gender of newborns, $F(6,38) = .544$, $p > .05$, and also the interaction between the presented odorant, heart rate variation, and gender of newborns proved to be nonsignificant, $F(12,78) = 1.074$, $p > .05$.

Therefore, the gender of newborns is disregarded in the following boxplots depicting the heart rate means for each of the stimuli in three consecutive trials. In Graph 9, the 10 s heart rate means of newborns in the first trial are shown in relation to the stimuli presented.

Graph 9: Representing the heart rate means based on the presented odorant in the first trial



Further, repeated planned contrasts showed a significant difference between presented odorants across repeated measures circa 40-50s after the odorant presentation, with $F(2,43)$ ranging between 1303.13 and 1332.39, $p < .01$.

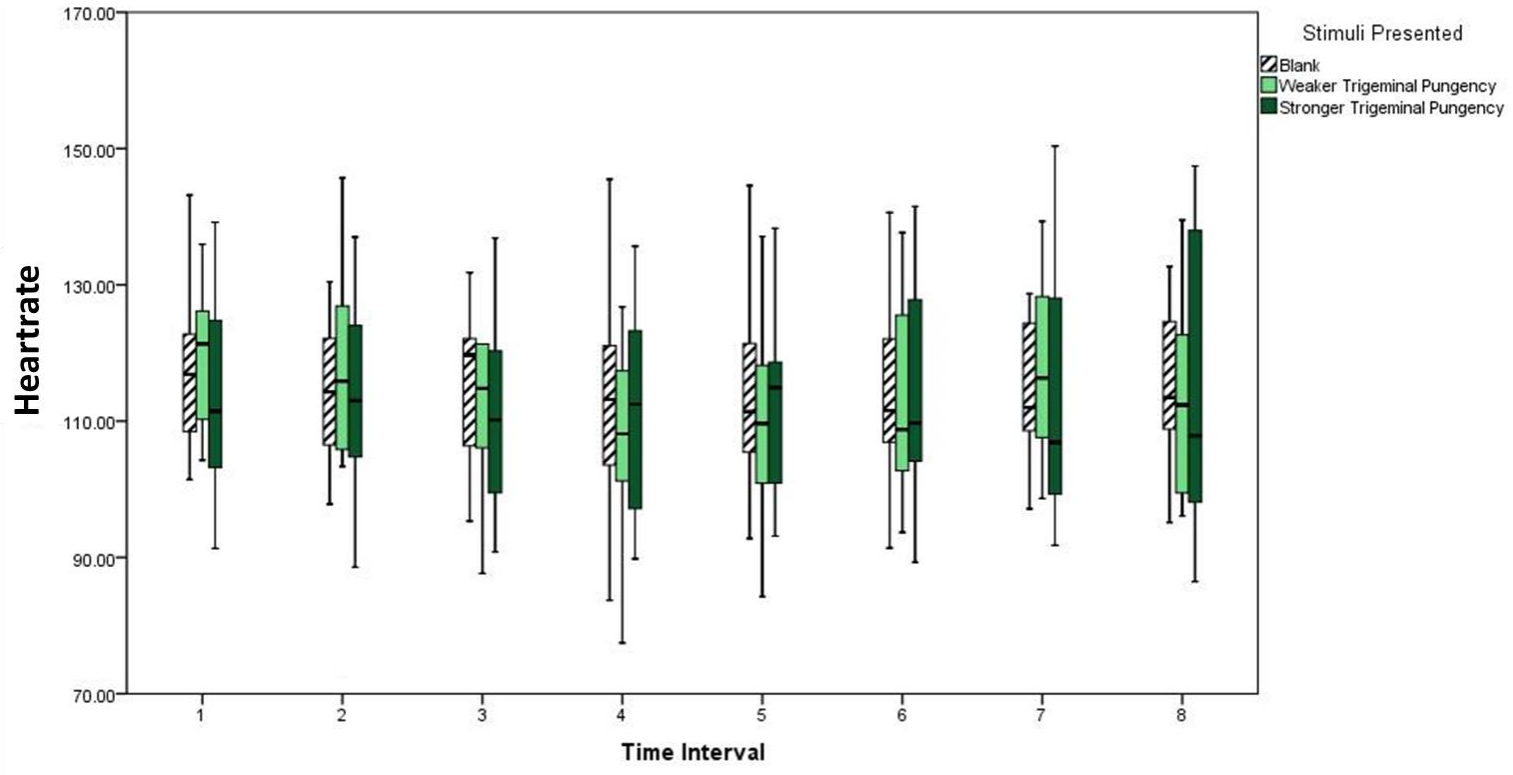
Paired samples t-tests have shown that there was a significant difference between the first repeated measure on the one hand, and the sixth and seventh one, on the other hand, for the blank stimulus only, $t(41) = 2.619$, $p < .05$ and $t(40) = 2.004$, $p = .052$, respectively. However, no such difference was found for the relatively mild or relatively strong trigeminal stimulants. Finally, there was no difference in heart rate among the odour groups within the sixth and seventh repeated measure, respectively.

Second Trial

Multivariate tests showed a significant effect of the within-subject variable, Pillai's Trace = .363, $F(7,34) = 2.764$, $p < .05$, its significant interaction with baseline heart rate, Pillai's Trace = .356, $F(7,34) = 2.683$, $p < .05$.

However, the interaction of the presented odorant with the course of heart rate variation was not significant, Pillai's Trace = .272, $F(7,34) = .788$, $p > .05$, as were the interactions between the heart rate variation and the gender of newborns, Pillai's Trace = .154, $F(7,34) = .826$, $p > .05$, and the interactions between the presented odorant, heart rate variation, and gender of newborns, Pillai's Trace = .188, $F(14,70) = .520$, $p > .05$. In Graph 10, the 10 s heart rate means of newborns in the second trial are shown in relation to the stimuli presented.

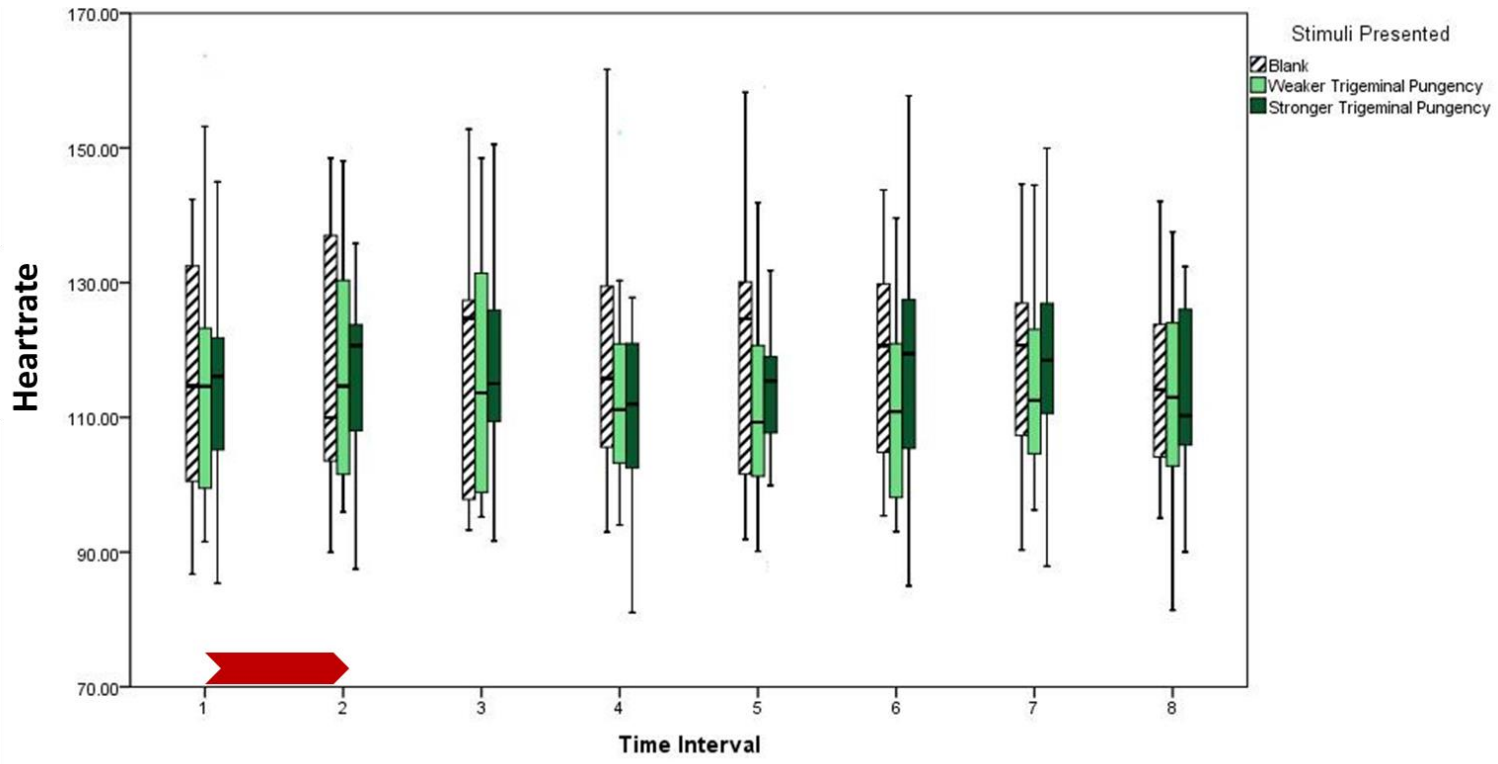
Graph 10: Representing the heart rate means based on the presented odorant in the second trial



Third Trial

Here, were no significant findings across all measured variables. Multivariate tests showed a non-significant effect of the within-subject variable, Pillai's Trace = .099, $F(7,30) = .472$, $p > .05$, its interaction with baseline heart rate, Pillai's Trace = .103, $F(7,30) = .495$, $p > .05$. Also, the interaction of the presented odorant with the course of heart rate variation was not significant, Pillai's Trace = .394, $F(14,62) = 1.087$, $p > .05$ as were the interactions between the heart rate variation and the gender of newborns, Pillai's Trace = .303, $F(7,30) = 1.860$, $p > .05$, and the interactions between the presented odorant, heart rate variation, and gender of newborns, Pillai's Trace = .310, $F(14,62) = .814$, $p > .05$. In Graph 11, the 10 s heart rate means of newborns in the third trial are shown in relation to the stimuli presented.

Graph 11: Representing the heart rate means based on the presented odorant in the third trial



2.5. Discussion

The present study addressed the responsiveness to olfactory stimuli in newborns. Namely, we sought to explore whether unfamiliar odours with contrasted trigeminal component would elicit differential heart rate variations indicative of arousal magnitude. Our findings indicate that newborns' reactions varied in relation to the odour's nasal trigeminal pungency in the first trial, where a significant interaction of the odour with the course of heart rate variation was found.

However, contrary to our assumptions, post-hoc analyses have shown a significant difference between the first repeated measure and the two repeated measures in which a change occurred (i.e., the sixth and the seventh one) in the blank stimulus only, with heart rate exhibiting a significant drop. In the relatively mild and strong trigeminal stimulants no such effect was found. This finding is only relevant to the first trial. This is in contrast to previous studies (e.g., Allen et al., 1979; Tuladhar et al., 2005) where a sudden trigeminal irritation elicited raised heart rate frequencies indicative of an arousal magnitude in newborns.

Based on our findings, we hypothesise that the slow, steady, and nonsignificant increase of heart rate in those newborns who were presented with odours stimulating the trigeminal nerve more could be ascribed to newborns holding their breath when being presented with an odour causing trigeminal irritation. In contrast, odours with a mild trigeminal component resulted in a steeper increase in heart rate, although this was not significant either. This may be, partly at least, caused by differential inhalation in response to mild and strong trigeminal stimulants. Changes in breathing patterns and mode of inhalation in children and adults alike in response to unpleasant olfactory stimulation are well established. Known as "sniff suppression", this phenomenon refers to sampling of unpleasant or strong odours using moderate to small sniffs, compared to pleasant or weak smells, which are inhaled more vigorously with larger sniffs (e.g., Mainland & Sobel, 2006; Johnson et al. 2003; Warren et al. 1994). In general, malodours, but not nonmalodours elicit anticipatory responses consisting in greater sniff suppression (e.g., Tourbier & Doty 2007).

Therefore, it has previously been thought to be largely a matter of conscious cognitive modulation by judgment. However, more recent studies emphasize its reflex-like aspect, which possibly protects airways from potentially noxious stimuli (e.g., Johnson et al. 2004). This has even found use in the Sniff Magnitude Test, which is a method of evaluating olfactory function by means of changes of air pressure in the nose after sniffing an unpleasant odour (Frank et al. 2003). If the trigeminal intranasal system indeed acts as a sentinel of the airways and the brain, reflexively inhibiting inhalation of potentially hazardous substances, then one would expect that unfamiliar strong trigeminal stimulants should be perceived as largely unpleasant. Thus, greater sniff suppression would be expected in response to strong, compared to mild, trigeminal stimulants, resulting in reduced sampling of the odour. As seen in Soussignan et al. (2007), breathing patterns were significantly lower when newborn were presented with Butyric Acid (0.031% dilution) compared with Vanillin (0.31% diluent).

Furthermore, a strong trigeminal irritation has been shown to elicit neurological airway protection processes such as sneezing, coughing, or apnoea (Alarie 1966) and thus potentially even leading to bradycardia (Schaller, 2007). We are not suggesting here that our stimuli were strong enough to cause such a strong reaction, but still, their level of trigeminal irritation could have been strong enough to slower the newborns breathing patterns, thus reducing the amount of odour inhaled. As the magnitude of olfactory stimulation seems to correlate with the magnitude of autonomic response, at least as far as unpleasant stimuli are concerned, the reaction to strong trigeminal stimulants may be inhibited by the fact that they are not inhaled as vigorously as the relatively mild ones.

The relatively more rapid (but non-significant) heart rate increase of newborns who were presented with weak trigeminal stimulants could be explained along similar lines; the newborns were roused by the new smell and started actively sniffing and thus, increasing their heart rate frequencies above their initial value. It should be noted here, that all these changes occurred within the 10 beats per minute range. The design of our study was purposefully aimed at eliciting slight but still significant changes in newborns' state.

Newborns are reactive to sensory stimuli, though their responses may vary in time and be highly dependent on their behavioural status (for review see Lecanuet & Schaal, 1996). This could be evidenced in heart rate variation elicited by blank stimuli. The very slight initial raise in heart rate values in newborns was followed by slow return to the baseline levels. We hypothesise that either newborns were startled slightly by the movements in his or her surrounding — by the stimuli being put 1 cm from his or her nostrils — or started sniffing at the very slight, though still discernible odour of cotton emanating from all our stimuli.

The above mentioned explanation of the observed effects are hypothetical. At this stage, data from the electrical impedance curve and the pulse oximeter — both of which are explained briefly in the data section of this study — are not yet transformed to a format which could be put under further analysis. However, once completed, this could allow us to assess the actual breathing frequency of newborns and thus provide grounds for these assumptions.

No matter the reasons for the observed changes in heart rate variation in the first trial, we did find asymmetric processing of previously unknown odorants based solely on the physicochemical feature of odorants, namely their contrasting level trigeminal component. This processing could play a role in the induction of olfactory hedonics in humans. Moreover, as these the tactile-like precepts of trigeminal irritating along with the effects they elicit such as sneezing, coughing, and weeping are generally perceived as unpleasant, unfamiliar odorants causing a stronger trigeminal irritation could be, in the same context, experienced as less pleasant compared to those stimulating trigeminal nerve less. In fact, several odorants with strong trigeminal component are sources of health hazards (Doty & Cometto-Muniz 2003).

We did not observe significant changes in the heart rate variation in relation to the odours in the second and third trial. We hypothesize that this could be due to the fact that behavioural states of newborns were assessed by the attending physician only prior to the presentation. Once the recording stated, only those newborns who awoke were disregarded from the study. There are five behavioural states as defined by Prechtl (1974) and newborns tend to alternate them rather easily. Therefore, in the consequent trials, newborns could have been in other behavioural stages and, as

sensorily-elicited heart rate responses are highly state-dependent in newborns (for review see Ashton, 1973), this could explain why our data show no significant changes in heart rate variations. We are planning to use the visual recordings of newborns to assess their behavioural responses — such as opening or closing of the mouths, eye movements and movements of head and limbs — to assess the behavioural stages of newborns in later trials.

Another factor that seems to have an effect on heart rate variation is the perceived pleasantness of odours (Bensafi et al., 2002a, 2002b; Delplanque et al., 2009). As in our study, we were focusing on the level the odours stimulate the trigeminal nerve, we had in each group of stimuli (relatively strong vs. relatively mild trigeminal irritants), two odours that are perceived by adult raters as pleasant and two that are perceived as unpleasant. Therefore, the effect of perceived hedonicity driven by other factors than those resulting from the trigeminal component itself could have been disregarded in our performed analysis. In future, these odours could be analysed each separately and thus the effect of both the trigeminal irritation and other aspects of perceived hedonicity on heart rate variations assessed.

No effect of the gender of newborn on elicited heart rate variation was found in our study. Olfactory system in humans, as well as in other vertebrates, seems to be sexually dimorphic with women exhibiting higher concentration of grey matter in several olfactory regions, namely the orbitofrontal cortex, and basal insular cortex (Garcia-Falgueras et al., 2006). Women outperform men in most olfactory tasks (detection, discrimination, and identification) though there is some discussion about whether sexual differences in olfactory abilities are due to women being more 'sensitive' to chemesthetic stimulations, or if these could be ascribed to females higher cognitive functioning (Radulescu & Mujica-Parodi, 2013). No matter the reason, these life-long differences are reported as early as within the first few hours after birth (Balogh & Porter, 1986), so some differences could have been expected in our sample as well. However, we did not observe any significant sex differences in newborn heart rate responsiveness to olfactory stimuli and thus the gender factor was excluded from the statistical analyses. Same results are reported by other studies on newborns where they also did not reveal any reliable effect of sex on the heart rate response (Soussignan et al., 1997).

The contradictory findings in studies regarding the gender effect in olfactory abilities in newborns could be explained in terms of differential sample sizes, and thus less explanatory power. Studies performed on newborns generally operate with smaller sample sizes (for further reference see Schaal, 2015) and therefore the effect of gender could be too weak to prove significant. In our study, we were planning to collect 60 newborns (F=30), however, due to circumstances outside our control, our final sample entailed only 50 (F=24) participants. Twenty-four females vs. twenty-six males could be too small a sample to detect a significant gender difference.

A possible limitation of our study was that the experimental room located in the maternity ward was not soundproofed. Audio stimuli could have affected the observed heart rate variations (Lecanuet & Schaal, 1996). However, these would have been expressed in the changed behaviour stage of newborns and thus will be potentially controlled for once the aforementioned assessment of videotapes is finished.

CONCLUSION

In sum, we feel that our significant findings relating to the first odorant presented are informative about the general responsiveness of newborns to unfamiliar odorants varying in their levels of trigeminal pungency. Especially given so low levels of stimuli's concentration, the newborns did on their heart rate level react asymmetrically to the odours presented based solely on their physicochemical structure. This observed asymmetry in processing of odours could point to the odorants' structure playing a role in the induction of olfactory hedonics.

Heart rate acceleration is a reliable marker of perceived unpleasantness of odours. However, contrary to our expectation and previous literature, in our study, the intense, relative to a mild, trigeminal odour did not elicit heart rate acceleration in newborns that could be indicative of an aversive reaction to unknown odorants based solely on the level they stimulate the trigeminal nerve. Thus, our preliminary findings do not support the hypothesis that trigeminal odorants would be — even without prior exposure to them — perceived as more unpleasant and therefore could help to avert individual from sources that might present a serious health hazard to him or her.

We feel that more research is needed providing us with better understanding about the effects trigeminal stimulation has on newborns, not only because of the role it could play in the induction of olfactory hedonics but also because its effects on newborns are not yet understood. In the maternity ward, newborns, even the preterm ones, are surrounded with sources of strong trigeminal stimuli, often in forms of disinfectants, detergents, and adhesive removers and still their perception and reaction to these stimulations remain unclear.

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ATTACHMENTS

1. Attachment A

In this attachment, there are documents used in Pilot Study I and II. As both studies were using same procedure, just different measure of comparison — Nutrilon for Pilot Study I, Vanillin for Pilot Study II — we took the liberty to include only the information sheet for the Pilot Study I. Other than that Attachment A includes one written consent form and one questionnaire as same ones were used in both pilot studies.

All documents had this header.

VÝZKUM ČICHOVÝCH PREFERENCÍ NOVOROZENÝCH DĚTÍ

UK v Praze a VFN Gynekologicko-porodnická klinika

Attachment A; Information sheet for Pilot Study I

INFORMACE PRO ÚČASTNÍKY VÝZKUMU

Vážená paní, vážený pane,

rádi bychom Vás informovali o projektu zaměřeném na výzkum čichových preferencí novorozených dětí a chtěli bychom Vás tímto pozvat k účasti na pilotní studii k tomuto projektu, která má za úkol zjistit vnímání intenzity roztoků látek s různým stupněm jejich ředění v porovnání s kojeneckým mlékem Nutrilonem. Vnímání intenzity potřebujeme zjistit proto, abychom mohli vybrat stimuly pro studii na novorozených dětech, kde budeme zkoumat jejich autonomní a behaviorální reakce na vystavení odorantům, které dráždí trojklaný nerv silněji a slaběji. Pro celý projekt je naprosto stěžejní vybrat takové koncentrace látek, které jsou vnímány jako víceméně stejně intenzivní, abychom sledovanou reakci novorozenců mohli skutečně připsat dráždění trojklaného nervu a ne rozdílům v intenzitách čichových vzorků.

Cíl studie: Zhodnotit, nakolik se vybrané roztoky látek blíží svou vnímanou intenzitou k intenzitě kojeneckého mléka Nutrilonu.

Podmínky účasti

Ženy a muži ve věku 18 – 35 let, netrpící astmatem a bez akutních příznaků rýmy.

Co účast v projektu obnáší:

Určování intenzity pachových látek v místnosti. Účast ve výzkumu je zcela dobrovolná a v jakémkoliv okamžiku můžete spolupráci na výzkumu bez udání důvodu ukončit. Před zahájením studie budete požádán(a) o podpis Informovaného souhlasu.

Způsob nakládání s daty:

Veškeré získané údaje budou využity pouze pro výzkumné účely a je s nimi nakládáno v souladu s platnými zákony České republiky o ochraně osobních údajů. Získané údaje jsou zpracovány anonymně (tj. pod číselným kódem) a publikovány budou pouze celkové výsledky, nikoliv údaje o jednotlivcích.

Možná rizika:

Vzhledem k tomu, že se jedná o velmi nízké koncentrace látek – všechny koncentrace se pohybují na, nebo pod jejich prahovou hranicí stanovenou pro dospělé – zdravotní rizika nejsou známa. Pokud budete během pokusu pociťovat jakýkoliv fyzický či psychický nesoulad, prosím, neodkladně informujte výzkumníky a domluvíme se na dalším postupu.

Motivační odměna:

Jako kompenzaci za čas strávený projektem a možné nepohodlí bude účastníkům nabídnuta drobná věcná odměna.

Finanční podpora:

Projekt je podporován grantem GAČR (14-02290S).

Etické aspekty:

Projekt byl schválen Etickou komisí Přírodovědecké fakulty UK (http://web.natur.cuni.cz/flegr/irb.php). V případě stížnosti spojené s projektem můžete kontaktovat předsedu etické komise: Prof. RNDr. Jaroslav Flegr, CSc. (flegr@cesnet.cz).

Kontakt:

Hlavní řešitel: doc. Jan Havlíček, Ph.D., Přírodovědecká fakulta, Univerzity Karlovy
Kontakt: tel: 221 951 853, 736 737 951 | e-mail: jhavlicek@natur.cuni.cz
Adresa: Viničná 7, 128 00 Praha 2

Souhlasíte-li s účastí ve výše popsaném výzkumu, prosíme Vás o vyplnění přiloženého **informovaného souhlasu.**

Attachment A; Written consent for both pilot studies

Informovaný souhlas

Název studie: Výzkum čichových preferencí novorozených dětí

Jméno

Datum narození

1. Já, níže podepsaný/á, souhlasím se svou účastí ve studii. Je mi více než 18 let.

2. Byl (a) jsem podrobně informován (a) o cílech studie, o jejích postupech a o tom, co se ode mě očekává.

3. Potvrzuji, že splňuji podmínky účasti ve studii.

4. Byl (a) jsem informován (a) o tom, že svou účast ve studii mohu kdykoliv přerušit či odstoupit, aniž by to jakkoliv ovlivnilo moje další práva. Účast ve studii je dobrovolná.

5. Bylo mi sděleno, že získaná osobní data budou uchována s plnou ochranou důvěrnosti dle platných zákonů ČR. Při vlastním provádění studie mohou být osobní údaje použity pro výzkumné a vědecké účely pouze bez identifikačních údajů (anonymní data) nebo s mým výslovným souhlasem.

6. Porozuměl (a) jsem tomu, že identifikační údaje (např. jméno) nebudou zveřejněny.

7. Svým podpisem stvrzuji, že nemám námitek proti tomu, aby mnou poskytnutá data byla za výše uvedených podmínek anonymně použita pro vědecko-výzkumné účely.

Podpis: Datum:

Attachment A; Questionnaire for both pilot studies

ID:	**věk:**	**Kouříte?** ANO/NE	Kolik cigaret/den:	Kouření poslední 2 hod.? ANO/NE
Máte nyní alergii, rýmu či jiné onemocnění ovlivňující čich: ANO/NE				Jaké?
Ženy: užíváte hormonální antikoncepci? ANO/NE				Uveďte název:
Lidé se liší tím, jak citlivě vnímají různé pachy. Například někdo určitý pach ani nezaznamená, zatímco někdo jiný cítí i velmi slabé pachy. **Jak citlivě vnímáte pachy Vy?** *(prosím zakroužkujte)* mnohem méně citlivě než ostatní lidé — méně citlivě než ostatní lidé — stejně citlivě jako ostatní lidé — citlivěji než ostatní lidé — mnohem citlivěji než ostatní lidé				
Obnášelo některé Vaše zaměstnání pravidelnou a dlouhodobou práci s chemikáliemi? *Prosím zakroužkujte:* ANO / NE - **Pokud ANO, jakou?** *(popište několika slovy)*				
Obnášelo některé Vaše zaměstnání pravidelný a dlouhodobý pobyt v prašném prostředí? *Prosím zakroužkujte:* ANO / NE - **Pokud ANO, jakou?** *(popište několika slovy)*				

Pokyny: V následujícím dotazníku budete hodnotit čichové vzorky. Je důležité, abyste všechny vzorky hodnotil/a v pořadí, které Vám sdělí výzkumník. Vnímanou intenzitu čichových vzorků budete porovnávat s 1 % roztokem vanilinu a na třech, po sobě jdoucích analogových škálách zaznamenávat, o kolik se podle Vás vzorky liší. Prosíme Vás o čitelné poznamenání kódu vzorku.

V případě, že je vzorek tak slabý, že ho vůbec necítíte, napište prosím jeho kód do kolonky Necítím.

2. Attachment B

In this attachment, there are documents used in Main Study. Therefore, it includes: Information sheet for Main Study, the written consent form and the questionnaire with the last two pages being filled by attending doctors.

All documents had this header.

VÝZKUM ČICHOVÝCH PREFERENCÍ NOVOROZENÝCH DĚTÍ

UK v Praze a VFN Gynekologicko-porodnická klinika

Attachment B; Information sheet for Main Study

Informace pro maminky

Vážená paní,

rádi bychom Vás informovali o studii zaměřené na vývoj čichových preferencí u novorozených dětí a chtěli bychom Vás tímto pozvat k účasti na ní.

Je prokázáno, že čichové preference mají významný vliv na naše chování v celé řadě oblastí, jako je vztah matky a dítěte či stravovací návyky. Převládá přitom názor, že si své čichové preference utváříme tak, že si vůně a pachy spojujeme s jinými libými či nelibými podněty (zážitky), následkem čehož se tato libost a nelibost přenáší i na tyto – původně neutrální – čichové podněty. Je však možné, že některé pachy jsou příjemnější než jiné „samy o sobě“, aniž by s nimi člověk měl předchozí zkušenost. To je dáno mírou, s jakou stimulují takzvaný trojklaný nerv, který zprostředkuje pocity chladu, tepla, šimrání v nose a další. Obracíme se proto s prosbou o spolupráci na Vás, jako maminku novorozeného miminka, které má s vůněmi a pachy zatím velmi málo zkušeností. Výsledky tohoto výzkumného projektu nám umožní hlubší pochopení vzniku čichových preferencí. Vzhledem k tomu, že čichové preference hrají podstatnou roli při formování stravovacích návyků, výsledky výzkumu tak bude možné využít pro porozumění vzniku poruch příjmu potravy či obezity.

Cíle projektu:
Testovat reakce novorozenců na aromatické látky s různou mírou aktivace trojklaného nervu.

Podmínky účasti
Novorozenci ve věku 3-5 dní, narozeni fyziologickým porodem bez zdravotních potíží.

Co účast v projektu obnáší:
Průběh testování byl navržen tak, aby byl pro Vás a Vaše miminko maximálně bezpečný a pohodlný. Den před zahájením studie budete požádána o podpis Informovaného souhlasu a vyplnění krátkého dotazníku ohledně průběhu těhotenství a porodu. Účast ve výzkumu je zcela dobrovolná a v jakémkoliv okamžiku můžete spolupráci na výzkumu bez udání důvodu ukončit.

Lékař i Vy sama budete přítomna po celou dobu testování, které potrvá nejvýše 15 min., z toho 10 min. potřebujeme na přípravu testování a 5 min. na testování samotné. Testování bude probíhat poté, co miminko po nakojení/nakrmení usne. Nad postýlku umístíme látková „nebesa“, která tlumí rušivé podněty zvenčí a na jejichž rámu je připevněna kamera, a dále přístroj na snímání srdečního tepu a dechu. Poté, co doktorka umístí na miminko čidla srdečního tepu a dechu, zapneme kameru a budeme postupně na tyčince k nosním dírkám miminka přikládat 3 čichové podněty: jeden, který stimuluje trojklaný nerv méně, jeden, který jej stimuluje více, a jeden kontrolní podnět bez pachu. Pro každé miminko budou náhodně vybrány dvě látky z těchto osmi látek:

- vanilin (1000ppm8 roztok), který je cítit po vanilce
- izoamyl acetát (1000ppm roztok), který je cítit po banánech
- karvon (1000ppm roztok), který je cítit po mátě
- eukalyptol (100ppm roztok), který je cítit po eukalyptu
- trimetylamin (10ppm roztok), který je cítit po rybě
- kyselina máselná (100ppm roztok), která je cítit po žluklém másle
- kyselina thioglykolová (100ppm roztok), která je cítit po zkažených vejcích
- merkaptoetanol (10ppm roztok), který je cítit po drůbeži

Všechny uvedené látky jsou zdravotně zcela nezávadné a byly v předchozích studiích testovány u novorozenců či kojenců.

Přitom budeme sledovat a na kameru zaznamenávat změny mimiky, srdečního tepu a dechu spícího miminka. Následně budete požádána o vyplnění dotazníku ohledně kojení. Tím bude testování u konce a v případě zájmu Vám předáme záznam nahrávky Vašeho miminka.

Způsob nakládání s daty:
Veškeré získané údaje budou využity pouze pro výzkumné účely a je s nimi nakládáno v souladu s platnými zákony České republiky o ochraně osobních údajů. Získané údaje jsou zpracovány anonymně (tj. pod číselným kódem) a publikovány budou pouze celkové výsledky, nikoliv údaje o jednotlivcích.

Možná rizika:
Žádná rizika spojená s účastí nejsou známa. V případě, že by během testování došlo k jakýmkoli komplikacím např. k výrazné změně v dechové frekvenci či k probuzení miminka, bude testování okamžitě ukončeno a miminko předáno do péče lékaře přítomného u testování.

Motivační odměna:
Jako kompenzaci za čas strávený projektem a možné nepohodlí budou zákonným zástupcům nabídnuty stravenky, které lze uplatnit na nákup stravy nebo oblečení pro miminko.

Finanční podpora:

[8] parts-per million, 1% je rovno 10 000ppm

Projekt je podporován grantem GAČR (14-02290S).

Etické aspekty:
Projekt byl schválen etickou komisí 1. lékařské fakulty UK. V případě stížnosti spojené s projektem můžete kontaktovat předsedu etické komise: MUDr. Josef Šedivý, CSc. (tel. 224 964 131).

Hlavní řešitel:
doc. Jan Havlíček, Ph.D., Přírodovědecká fakulta, Univerzity Karlovy
Kontakt: tel: 221 951 853736 737 951 | e-mail: jhavlicek@natur.cuni.cz
Adresa: Viničná 7, 128 00 Praha 2
Kontaktní osoba:
Jiřina Boušová
Tel.: 607 608 962
e-mail : jirinabousova@gmail.com

Attachment B; Written consent form for Main Study

Informovaný souhlas

Identifikační kód (vyplní výzkumník):_____

1. Byla jsem podrobně informována o cílech studie, o jejích postupech a o tom, co se ode mě a nezletilé osoby očekává.
2. Byla jsem informována o tom, že svou účast ve studii mohu kdykoliv přerušit či odstoupit, aniž by to jakkoliv ovlivnilo moje další práva. Účast ve studii je dobrovolná.
3. Bylo mi sděleno, že získaná osobní data budou uchována s plnou ochranou důvěrnosti dle platných zákonů ČR. Při vlastním provádění studie mohou být osobní údaje použity pro výzkumné a vědecké účely pouze bez identifikačních údajů (anonymní data) nebo s mým výslovným souhlasem.
4. Porozuměla jsem tomu, že identifikační údaje (např. jméno) nezletilého účastníka výzkumu nebudou zveřejněna.
5. Svým podpisem stvrzuji, že jako zákonný zástupce souhlasím s účastí svěřené nezletilé osoby ve studii. Nemám námitek proti tomu, aby mnou poskytnutá data byla za výše uvedených podmínek anonymně použita pro vědecko-výzkumné účely.

V Praze dne:

Jméno a příjmení účastníka výzkumu:

Jméno a příjmení zákonného zástupce:

Podpis zákonného zástupce:

Jméno a příjmení výzkumníka, který přijímal informovaný souhlas:
Jiřina Boušová
Podpis výzkumníka:

Attachment B; Questionnaire for Main Study

Identifikační kód (vyplní výzkumník): _____

Datum:_____

Do rukou se Vám dostal dotazník, který je zaměřen na zjištění základních osobnostních charakteristik, údajů o porodu a o Vašem dítěti, o Vašich pocitech a myšlenkách během posledního měsíce a o Vašich čichových preferencích.

Prosíme o pravdivé zodpovězení následujících dotazů. Pokud na některou z uvedených otázek nechcete odpovědět, raději ji přeskočte, než abyste uvedla nepravdivý údaj. Celý dotazník je zcela anonymní. Zaručujeme se, že všechny získané údaje budou použity pouze k vědeckým účelům a nebudou poskytovány třetím osobám.

Oddíl 1: Čichový test

V následujícím dotazníku budete hodnotit čichové vzorky. Je důležité, abyste vzorky hodnotila v pořadí, které Vám sdělí výzkumník. V případě, že je vzorek tak slabý, že jej necítíte vůbec, zakroužkujte prosím „necítím“ a dále jej již nehodnoťte. Příjemnost vzorku hodnoťte na škále v rozmezí od -3 do 3 (0 = neutrální). Pro hodnocení intenzity a známosti použijte škály od 1 do 7. Nezapomeňte prosím vždy poznamenat kód vzorku!

Vzorek číslo:		necítím

Jak byste tento pach popsala?

1) Nakolik ve Vás pach vyvolává pálivý pocit?							2) Nakolik ve Vás pach vyvolává chladivý pocit?						
1 vůbec	2	3	4	5	6	7 velmi	1 vůbec	2	3	4	5	6	7 velmi

3) Kterými z následujících charakteristik byste vzorek popsala? Prosím zaškrtněte libovolný počet charakteristik, které se podle Vás hodí.

pronikavý způsobuje pálení	vyvolává svíravý pocit vyvolává pocit tepla	způsobuje mravenčení způsobuje kýchání	sladký slaný
způsobuje bolest ostrý	způsobuje škrábání způsobuje šimrání	vyvolává chladivý pocit vyvolává pocit svěžesti	hořký či trpký kyselý

4) Jak příjemný je pro Vás tento pach? (-3 = velmi nepříjemný, 3 = velmi příjemný)

-3	-2	-1	0	1	2	3

5) Jak intenzivní je pro Vás tento pach? (1 = vůbec, 7 = velmi)

1	2	3	4	5	6	7

6) Jak známý je pro Vás tento pach? (1 = zcela neznámý, 7 = velmi dobře známý)

1	2	3	4	5	6	7

7) Co by podle Vás mohlo být zdrojem tohoto pachu, popř. co Vám tento pach připomíná?

.....

.....

Vzorek číslo:		necítím

Jak byste tento pach popsala?

1) Nakolik ve Vás pach vyvolává pálivý pocit?							2) Nakolik ve Vás pach vyvolává chladivý pocit?						
1 vůbec	2	3	4	5	6	7 velmi	1 vůbec	2	3	4	5	6	7 velmi

3) Kterými z následujících charakteristik byste vzorek popsala? Prosím zaškrtněte libovolný počet charakteristik, které se podle Vás hodí.

pronikavý	vyvolává svíravý pocit	způsobuje mravenčení	sladký
způsobuje pálení způsobuje bolest	vyvolává pocit tepla způsobuje škrábání	způsobuje kýchání vyvolává chladivý pocit	slaný hořký či trpký
ostrý	způsobuje šimrání	vyvolává pocit svěžesti	kyselý

4) Jak příjemný je pro Vás tento pach? (-3 = velmi nepříjemný, 3 = velmi příjemný)

-3	-2	-1	0	1	2	3

5) Jak intenzivní je pro Vás tento pach? (1 = vůbec, 7 = velmi)

1	2	3	4	5	6	7

6) Jak známý je pro Vás tento pach? (1 = zcela neznámý, 7 = velmi dobře známý)

1	2	3	4	5	6	7

7) Co by podle Vás mohlo být zdrojem tohoto pachu, popř. co Vám tento pach připomíná?

.....
.....

Oddíl 2: Demografické údaje

Kolik je Vám let? .....

Jaký je Váš mateřský jazyk? ……..

Jaký je Váš rodinný stav?
svobodná
vdaná
rozvedená
vdova

Jakého stupně vzdělání jste doposud dosáhla?
základní škola
střední škola s výučním listem
střední škola s maturitou
vyšší škola
vysoká škola

Pokud studujete, kterou školu a na jakém stupni studujete?
.....

Jaká je výše současného čistého měsíčního příjmu Vaší domácnosti?
0 – 10.000,-
10.000 – 20.000,-
20.000 – 35.000,-
35.000 – 50.000,-
50.000 a více

Prosím, uveďte Vaši váhu a výšku před otěhotněním:

Váha: .....kg Výška: …….....cm

Prosím, uveďte velikost Vaší podprsenky (např. 80B):

před otěhotněním …… nyní: ……

Vyznačte prosím, zda byl Váš způsob stravování v těhotenství:
běžný (jíte vše nebo téměř vše)
vegetariánský
veganský
jiný (různá specifická stravovací omezení jako je např. diabetická dieta)
Prosím, uveďte jaký:
.....

Jak často jste během svého těhotenství jedla níže uvedené aromatické potraviny:

	Méně jak jednou měsíčně	Jednou měsíčně	Několikrát za měsíc	Jednou týdně	Několikrát týdně	Obden	Denně
Kysané zelí							
Ředkvičky							
Nakládané ryby							
Feferonky							
Syrovou cibuli							
Česnek							
Celer							
Ocet							
Zrající sýry							
Jiné aromatické potraviny. Prosím, specifikujte:							

Jak často jste během svého těhotenství trpěla ranními nevolnosti?

Méně jak jednou měsíčně	Jednou měsíčně	Několikrát za měsíc	Jednou týdně	Několikrát týdně	Obden	Denně

Jak si myslíte, že se Vám během těhotenství změnily chuťové preference?

Vůbec	Skoro vůbec	Trochu	Středně	Docela silně	Silně	Velmi silně

Pokud ano, prosím uveďte, u kterých chutí došlo k největším změnám:

.....

Otěhotněla jste během používání hormonální antikoncepce? ANO / NE
Pokud ano, prosím uveďte značku antikoncepce a v kolikátém týdnu těhotenství jste ji vysadila:

.....

Užívala jste během těhotenství nějaké léky proti bolesti
(např. Acylpyrin, Aspirin, Ibuprofen, Nalgesin…)? ANO / NE
Pokud ano, prosím uveďte názvy léků a jejich dávkování:

.....

Užívala jste během těhotenství i jiné léky? ANO / NE
Pokud ano, prosím uveďte názvy léků a jejich dávkování:

.....
.....
.....

Vyznačte prosím, zda jste:
kuřačka
Pokud ano, prosím uveďte odhadem, kolik cigaret za den kouříte:

.....cigaret za den

bývalá kuřačka
Pokud ano, prosím uveďte odhadem, před kolika měsíci jste přestala a
kolik cigaret za den jste kouřila:

.....měsíci .....cigaret za den
nekuřačka

Užívala jste během těhotenství nějaké ilegální drogy? ANO / NE
Pokud ano, prosím uveďte jaké a odhadem a jak často:

.....

Pila jste během těhotenství alkohol? ANO / NE
Pokud ano, prosím uveďte jaký a odhadem jak často:

.....

Oddíl 3: Porod a Vaše dítě

Tento porod byl Váš:
první
druhý
třetí
čtvrtý a více

V případě, že se nejedná o Váš první porod, prosím uveďte datum svého předchozího porodu:
……

Trpěla jste během svého těhotenství nějakými potížemi / chorobami?
ANO / NE
Pokud ano, prosím uveďte jakými:

.....

Bylo během Vašeho těhotenství vzneseno podezření na nějaké vývojové vady?
ANO / NE
Pokud ano, prosím uveďte na jaké:

.....

Pohlaví dítěte: ŽENA / MUŽ

Pokud cítíte, že se během Vašeho těhotenství odehrálo cokoliv, co hodnotíte jako podstatné, případně zvláštní, budeme rádi, když nám o tom zde sdělíte více:

.....
.....
.....

Oddíl 4: Vaše pocity a myšlenky během posledního měsíce

Následující otázky jsou cíleny na Vaše pocity a myšlenky a na to, jak často jste je během posledního měsíce těhotenství zažívala. Po přečtení otázky vždy prosím zakroužkujte právě jednu z následujících variant.

Jak často jste byla během posledního měsíce rozrušená kvůli něčemu, co se nečekaně přihodilo?

Téměř nikdy	Zřídka	Občas	Celkem často	Velmi často

Jak často jste během posledního měsíce měla pocit, že nemáte důležité věci ve svém životě pod kontrolou?

Téměř nikdy	Zřídka	Občas	Celkem často	Velmi často

Jak často jste se během posledního měsíce cítila nervózní a ve stresu?

Téměř nikdy	Zřídka	Občas	Celkem často	Velmi často

Jak často jste během posledního měsíce věřila v to, že dokážete zvládnout své osobní problémy?

Téměř nikdy	Zřídka	Občas	Celkem často	Velmi často

Jak často jste během posledního měsíce měla pocit, že se věci dějí tak, jak byste si přála?

Téměř nikdy	Zřídka	Občas	Celkem často	Velmi často

Jak často jste během posledního měsíce měla pocit, že se nezvládnete vypořádat se všemi povinnosti?

Téměř nikdy	Zřídka	Občas	Celkem často	Velmi často

Jak často jste během posledního měsíce zvládala mít pod kontrolou to, co Vás rozčiluje?

Téměř nikdy	Zřídka	Občas	Celkem často	Velmi často

Jak často jste měla během posledního měsíce pocit, že věci dokážete snadno kontrolovat?

Téměř nikdy	Zřídka	Občas	Celkem často	Velmi často

Jak často jste byla během posledního měsíce rozzlobená kvůli věcem, které jste nemohla ovlivnit?

Téměř nikdy	Zřídka	Občas	Celkem často	Velmi často

Jak často jste během posledního měsíce měla pocit, že se obtíže hromadí tak, že je nedokážete překonat?

Téměř nikdy	Zřídka	Občas	Celkem často	Velmi často

DĚKUJEME ZA VÁŠ ČAS A ÚČAST VE STUDII!

Pokud souhlasíte, byli bychom rádi, kdyby následující informace vyplnil Váš ošetřující lékař / lékařka.

Způsob porodu?
vaginální porod klasický (hlavičkou vpřed)
vaginální porod nepravidelný (koncem pánevním, zadní poloha)

Vyznačte prosím jevy, které se týkaly porodu (i více položek):
vyvolaný porod (hormonálně, mechanicky)
aplikace analgetika pro zmírnění bolesti (např. Pethidin. Entonox)
epidurální anestézie
nastřižení hráze (tzv. epiziotomie)
natržení hráze
podání Oxytocinu během II. doby porodní
jiné ……..

Uveďte prosím všechny vážnější komplikace, které se týkaly porodu:

.....
.....
.....

V kolikátém týdnu a dnu těhotenství nastal porod? .....

Jak dlouho porod trval (od prvních kontrakcí po samotný porod dítěte)?

.....

Datum a přesný čas porodu?
Datum ..... Přesný čas …….....

Hodnota Apgar skóre dítěte? ……..

Váhu a délku dítěte

	Váha (gramy)	Délka (centimetry)
První den (váha po porodu)		
Druhý den		x
Třetí den		x
Čtvrtý den		x

3. Attachment C

In this attachment, there are 9 GML models. On each following page there is a table depicting 3 models (1-3; 4-6; 7-9) with their partial eta squares, below which there are SPPS details of each model in question. Based on these, model 9 was used for the analysis.

Attachment C; 1-3 GLM models and partial eta squares

Partial Eta Square	Model 1			Model 2			Model 3		
	Multivariate	Within-subject	Between-subject	Multivariate	Within-subject	Between-subject	Multivariate	Within-subject	Between-subject
Gender			.001			.001			.000
Odour			.049			.079			.071
Interval	.457	.110		0.516	.121		.473	.017	
Heart rate baseline			.727			.608			
Interval*gender *baseline	.125	.018		.095	.018				
Interval*odour*baseline	.078	.026		.101	.025				
Interval*gender*odour	.449	.065		.146	.065		.590	.053	
Interval*odour	.087	.029		.108	.027		.006	.101	
Interval*gender	.131	.019		.095	.018		.756	.011	
Interval*baseline	.449	.106		.505	.117				
Odour*gender			.005			.021			.054
Gender*baseline			.000			.003			
Odour*baseline			.051			.078			

model 1

```
GLM prvnideset druhydeset trestideset ctvrtydeset patydeset sestydeset sedmydeset osmydeset BY prvnilátka
pohlavimimi WITH BSprumer
  /WSFACTOR=HR_cas_interval 8 Polynomial
  /MEASURE=HR  /METHOD=SSTYPE(3)
  /SAVE=PRED SEPRED RESID ZRESID COOK LEVER
  /PLOT=PROFILE(HR_cas_interval*prvnilátka HR_cas_interval*pohlavimimi HR_cas_interval*prvnilátka*pohlavimimi)
  /PRINT=DESCRIPTIVE ETASQ OPOWER PARAMETER HOMOGENEITY LOF GEF
  /CRITERIA=ALPHA(.05)  /WSDESIGN= HR_cas_interval
  /DESIGN= prvnilátka pohlavimimi BSprumer pohlavimimi*prvnilátka BSprumer*pohlavimimi BSprumer*prvnilátka.
```

model 2

```
GLM prvnideset druhydeset trestideset ctvrtydeset patydeset sestydeset sedmydeset osmydeset BY prvnilátka
pohlavimimi WITH BSprumer
  /WSFACTOR=cas_inteval 8 Repeated
  /MEASURE=HR  /METHOD=SSTYPE(3)
  /SAVE=COOK LEVER
  /PLOT=PROFILE(cas_inteval*prvnilátka*pohlavimimi)
  /EMMEANS=TABLES(OVERALL) WITH(BSprumer=MEAN)
  /PRINT=ETASQ HOMOGENEITY
  /CRITERIA=ALPHA(.05)  /WSDESIGN= cas_inteval
  /DESIGN= prvnilátka pohlavimimi BSprumer pohlavimimi*prvnilátka BSprumer*pohlavimimi BSprumer*prvnilátka.
```

model 3

```
GLM BSprumer prvnideset druhydeset trestideset ctvrtydeset patydeset sestydeset sedmydeset osmydeset BY prvnilátka
pohlavimimi
  /WSFACTOR=cas_inteval 9 Simple
  /MEASURE=HR  /METHOD=SSTYPE(3)
  /SAVE=COOK LEVER
  /PLOT=PROFILE(cas_inteval*prvnilátka*pohlavimimi)
  /EMMEANS=TABLES(OVERALL)
  /PRINT=ETASQ HOMOGENEITY
  /CRITERIA=ALPHA(.05)  /WSDESIGN= cas_inteval
  /DESIGN= prvnilátka pohlavimimi pohlavimimi*prvnilátka.
```

Attachment C; 4-6 GLM models and partial eta squares

Partial Eta Square	Model 4			Model 5			Model 6		
	Multivariate	Within-subject	Between-subject	Multivariate	Within-subject	Between-subject	Multivariate	Within-subject	Between-subject
Gender			.002			.014			.061
Odour			.041			.006			.008
Interval	.435	.090		.142	.015		.438	.104	
Heart rate baseline			.704						.726
Interval*gender *baseline	.140	.023							
Interval*odour* baseline	.087	.022							
Interval*gender* odour	0.152	.059		.145	.054		.143	.061	
Interval*odour	.093	.025		.301	.106		.289	.104	
Interval*gender	.145	.024		.074	.010		.045	.006	
Interval*baseline	.427	.087					.429	.100	
Odour*gender			.001			.014			.005
Gender*baseline			.004						
Odour*baseline			.040						

model 4

```
GLM prvnideset druhydeset tretideset ctvrtydeset patydeset sestydeset sedmydeset osmydeset devatydeset BY prvnilátka pohlavimimi WITH BSprumer80
  /WSFACTOR=cas_interval 9 Simple
  /MEASURE=HR  /METHOD=SSTYPE(3)
  /SAVE=COOK LEVER
  /PLOT=PROFILE(cas_interval*prvnilátka*pohlavimimi)
  /EMMEANS=TABLES(OVERALL) WITH(BSprumer80=MEAN)
  /PRINT=ETASQ HOMOGENEITY
  /CRITERIA=ALPHA(.05)  /WSDESIGN= cas_interval
  /DESIGN= pohlavimimi*prvnilátka BSprumer80*pohlavimimi BSprumer80*prvnilátka prvnilátka pohlavimimi BSprumer80.
```

model 5

```
GLM prvnideset druhydeset tretideset ctvrtydeset patydeset sestydeset sedmydeset osmydeset devatydeset BY prvnilátka pohlavimimi
  /WSFACTOR=cas_interval 9 Simple
  /MEASURE=HR  /METHOD=SSTYPE(3)
  /SAVE=COOK LEVER
  /PLOT=PROFILE(cas_interval*prvnilátka*pohlavimimi)
  /EMMEANS=TABLES(OVERALL)
  /PRINT=ETASQ HOMOGENEITY
  /CRITERIA=ALPHA(.05)  /WSDESIGN= cas_interval
  /DESIGN= pohlavimimi*prvnilátka prvnilátka pohlavimimi.
```

model 6

```
GLM prvnideset druhydeset trestideset ctvrtydeset patydeset sestydeset sedmydeset osmydeset BY prvnilátka pohlavimimi WITH BSprumer
  /WSFACTOR=cas_interval 8 Polynomial
  /MEASURE=HR  /METHOD=SSTYPE(3)
  /SAVE=COOK LEVER
  /PLOT=PROFILE(cas_interval*prvnilátka*pohlavimimi)
  /EMMEANS=TABLES(OVERALL) WITH(BSprumer=MEAN)
  /PRINT=ETASQ HOMOGENEITY
  /CRITERIA=ALPHA(.05)  /WSDESIGN=cas_interval
  /DESIGN=BSprumer prvnilátka pohlavimimi prvnilátka*pohlavimimi.
```

Attachment C; 7-9 GLM models and partial eta squares

Partial Eta Square	Model 7			Model 8			Model 9		
	Multivariate	Within-subject	Between-subject	Multivariate	Within-subject	Between-subject	Multivariate	Within-subject	Between-subject
Gender			.061			.061			.042
Odour			.008			.008			.003
Interval	.438	.104		.438	.104		.414	.083	
Heart rate baseline			.726			.726			.704
Interval*gender									
*baseline									
Interval*odour*baseline									
Interval*gender*odour	.143	.061		.143	.061		.146	.057	
Interval*odour	.289	.104		.289	.104		.299	.112	
Interval*gender	.045	.006		.045	.006		.080	.011	
Interval*baseline	.429	.100		.429	.100		.406	.080	
Odour*gender			.005			.005			.001

model 7

```
GLM prvnideset druhydeset trestideset ctvrtydeset patydeset sestydeset sedmydeset osmydeset BY prvnilátka pohlavimimi WITH BSprumer
/WSFACTOR=cas_interval 8 Repeated
/MEASURE=HR/METHOD=SSTYPE(3
/SAVE=COOK LEVER
/PLOT=PROFILE(cas_interval*prvnilátka*pohlavimimi)
/EMMEANS=TABLES(OVERALL) WITH(BSprumer=MEAN)
/PRINT=ETASQ HOMOGENEITY
/CRITERIA=ALPHA(.05) /WSDESIGN=cas_interval
/DESIGN=BSprumer prvnilátka pohlavimimi prvnilátka*pohlavimimi.
```

model 8

```
GLM prvnideset druhydeset trestideset ctvrtydeset patydeset sestydeset sedmydeset osmydeset BY prvnilátka pohlavimimi WITH BSprumer
/WSFACTOR=cas_interval 8 Simple(1)
/MEASURE=HR/METHOD=SSTYPE(3)
/SAVE=COOK LEVER
/PLOT=PROFILE(cas_interval*prvnilátka*pohlavimimi)
/EMMEANS=TABLES(OVERALL) WITH(BSprumer=MEAN)
/PRINT=ETASQ HOMOGENEITY
/CRITERIA=ALPHA(.05) /WSDESIGN=cas_interval
/DESIGN=BSprumer prvnilátka pohlavimimi prvnilátka*pohlavimimi.
```

model 9

```
GLM prvnideset druhydeset tretideset ctvrtydeset patydeset sestydeset sedmydeset osmydeset devatydeset BY prvnilátka pohlavimimi WITH BSprumer80
/WSFACTOR=cas_interval 9 Simple(1)
/MEASURE=HR/METHOD=SSTYPE(3)
/SAVE=COOK LEVER
/PLOT=PROFILE(cas_interval*prvnilátka*pohlavimimi)
/EMMEANS=TABLES(OVERALL) WITH(BSprumer80=MEAN)
/PRINT=ETASQ HOMOGENEITY
/CRITERIA=ALPHA(.05) /WSDESIGN=cas_interval
/DESIGN=BSprumer80 prvnilátka pohlavimimi prvnilátka*pohlavimimi.
```