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Infrared Thermal Imaging in Affective Neuroscience: Insights to the Self from the Peripheral Nervous System.

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Chapter 1

1. General Introduction

1.1. The Biological Origin of a Temperature Change in Thermal Infrared Imaging

Through hearing, smell, touch, vision, and taste the central nervous system monitors the exterior environment (Damasio & Carvlho, 2014). Neural maps of exteroceptive awareness generate instinctual physiological adaptations or ‘action programs’ in response to environmental demands. Successful exteroceptive detection leads to corrections in cognition (for example focused attention), modifications in visceral and internal milieu (for example heart rate increase and hormonal secretion) as well as changes in striated muscles (for example facial expression and running) that aim at restoring safety in both social and biological sense. The human face conveys the strongest communicative tool in social interaction (Ekman 1992) where phylogenetic shifts in neural regulation of the autonomic nervous system foster a variety of emotional displays and behaviours (Porges, 2001). Autonomic adaptation carries its own physiological thermal print and by harnessing the power given by homeostatic balance, distinctions can be made between arousal and parasympathetic restoration.

Thermal Imaging constitutes a novel approach in the arena of psychophysiology, where infrared light emitted naturally by the surface of the skin can be used for physiological observations of affective nature. Functional infrared thermal imaging (fITI) bases the majority of observations on the thermal print of the face. The rich underlying vasculature makes the face an ideal candidate for monitoring peripheral physiology as changes in heart rate, muscular adaptation and epinephrine release in the blood stream are depicted in specific facial regions with unique temperature elements (Ioannou, Gallese, Merla, 2013). Negative emotions such as fear (Nakayam, Goto, Kuraoka, Nakamura, 2005) and guilt (Ioannou et al., 2013) have been associated with a decrease in nose temperature, a phenomenon associated with subcutaneous vascular constriction, evident on the extremities of the body (nose, ears, fingers, paws, tail). On the contrary, social interaction (Ioannou et al., 2014) and sexual arousal (Hahn et al., 2012) have been associated with a temperature increase on the majority of facial regions, probably signalling the felt emotion. Moreover, in occasions of distress (such as mental workload or startle) perspiration pores increase (Pavlidis, et al., 2012) leading to a temperature decrease on the upper lip, whereas the peri-orbital region of the ocular cavity shows the opposite pattern, an increase in temperature (Pavlidis, Levine, Baukol, 2001). Authors suggest that the local musculature that surrounds the eyes is responsible for the temperature increase of the peri-orbital region facilitating rapid saccades in case of a perceived threat. The maxillary area (or the upper lip) although not among the regions of increased emotional sweating (such as the axillae, palms, and soles of the feet) shows a positive correlation with the fingers in response to startles. Thus inferences can be made of emotional arousal by observing the thermal signature of the upper lip (Shastri, Merla, Tsiamyrtzis, Pavlidis, 2009). In conclusion, the face can be used to observe holistic autonomic responses deriving from the endocrine system and nerve impulses of the vagus to the heart.

1.2. Potentialities and Limits of Thermal Imaging.

Unlike other physiological techniques thermal imaging has certain advantages and disadvantages. Conventional physiological measurements limit the way biological data are collected since they require restriction of the subject's movements (Nakayama, Goto, Kuraoka & Nakamura 2005; Kuraoka & Nakamura, 2011) or by invasive implantation of radio telemetric probes for autonomic monitoring (Vianna & Carrive, 2005). To the extent these methodologies need direct contact with the body, they limit the nature of the experimental paradigm. Moreover, the use of temperature sensors on the skin is not an option as they get detached through contact, cover a small surface area and their pressure on the skin can induce changes of regional blood flow affecting physiological recordings (Nakayama et al., 2005).

Functional Infrared Thermal Imaging (fITI) is a highly sensitive and versatile technique that converts infrared light into temperature, allowing wireless monitoring of the participant (Ring & Ammer, 2012). The simplicity of fITI use for physiological monitoring makes it particularly useful in experimental designs that resemble real life situations in which liberty of movement is essential. For example, self-conscious emotions like guilt are challenging to study as it is hard to substitute the social component with virtual (e.g. films, photographs) stimulus presentation. Furthermore, established physiological recording tools, because of their body contact, interfere with the experimental procedure as they constantly remind the participant that is being monitored. The non-invasive nature of fITI makes it ideal for ecological experimental designs as well as for sensitive population groups such as individuals with disabilities and infants that cannot express their emotions verbally (Nakanishi, Imai-Matsumura 2008). Its reliability has been examined simultaneously with gold standard physiological methods such as galvanic skin response (GSR) (Kuraoka & Nakamura, 2011; Pavlidis et al, 2012) laser Doppler flowmetry (Kistler, Mariazouls, & Berlepsch, 1998) and polygraph testing (Pavlidis, Eberhardt, & Levine, 2002). Compared to GSR, Infrared Thermal Imaging provides equal or even better detection power among affective states. Variation of stimulus intensity is evident in facial temperature, whereas with GSR each stimulus, irrespective of its intensity, produces the same signal (Kuraoka & Nakamura, 2011). In addition to its hypersensitivity drawback, GSR electrodes can loose conductivity over time (Levenson, 1988), signal changes can occur spontaneously during baseline (Laine, Spitler, Mosher, & Gothard, 2009) and even result from arm movements towards a rewarded stimulus (Amiez, Procyk, Honore, Sequeira, & Joseph 2003). Nevertheless, the sluggish nature of facial temperature change makes infrared imaging not a good candidate for experiments that are concerned about the temporal latency of an emotional response. At the best-case scenario a significant temperature change can be observed within 10 s, whereas with GSR within 3 s (Kuraoka & Nakamura, 2010).

1.3. The Psychophysiology of Self-Conscious Emotions and Adult Psychopathology

The verbal description of emotional sensations has often been used by psychological research to understand and differentiate among emotional experiences (Cacioppo, Gardner, & Berntson, 1999; LeDoux, 1996). Twenty years later the situation seems not much different with self-reports posing in top scientific journals; with the only exception that recalls have been replaced by beautifully digitised bodily maps (Nummenmaa, Glerean, Hari, & Hietanen, 2013). The reason for this phenomenon is that emotions are not only mental; they are also embodied (Larsen, Bernston, Poehlmann, Ito & Cacioppo, 2008). People use a variety of metaphorical depictions to describe their emotional experience referring to sensations such as butterflies in their stomach to express anxiety and blood boiling to mark anger. Damasio & Carvalho (2013) categorize these descriptions as ‘feelings’ which are sensed interoceptively on the body and mapped by the central nervous system as mental experiences that accompany the altering body states (Damasio, 1999). Nonetheless only some aspects of emotional experiences can be verbally reported (Bradley, 2000; Lang, 1971). Evidence from neuroscience (Tranel &

Damasio, 2000) as well as social psychology states that emotions can occur in the absence of feelings (Winkielman, Berridge, & Wilbarger, 2005), which are essential in order for verbal self-reports to take place on emotional experience. Self-reports can only modestly grasp the true element of a bodily emotional experience and their associated behavioral aspects (Bradley & Lang, 2000a; but see Mauss, Levenson, McCarter, Wilhelm, & Gross, 2005). Moreover, although facial expression may depict an emotion, there are certain emotions that are not accompanied by any specific facial action (Cacioppo & Petty, 1982). Thus, research in the psychophysiology of emotions aims to clarify and complement self-reports of emotions by investigating physiological marks of embodied experiences. Moreover in order to create a more detailed map of the feelings sensation an emotional response needs to be decomposed into its physiological subparts (e.g. temperature, heart rate, sweating) with the use of physiological methods in order to clarify to what extent can we trust self-reports on interoception (Cacioppo & Petty, 1987).

Distinctive physiological patterns accompany emotional experiences even if they belong to the same thematic emotional sphere (e.g. disgust, fear, anger) (Cacioppo, Berntson, Larsen, Poehlmann, Ito, 2000; Kreibig, 2010). Distinctions between varieties of emotional states can be made by looking not only at the different physiological elements (e.g. heart rate, skin conductance and temperature) but also by measuring their intensity such as heart rate acceleration or deceleration (Levenson, 1992). Nevertheless the crucial question to ask in the psychophysiology of emotions is not which distinctive physiological pattern accompanies different types of emotion, but under which circumstances do this situation-specific physiological patterns occur (Larsen, Bernston, Poehlmann, Ito & Cacioppo, 2008). The reason for this is that physiological activation alters the intensity and not the nature of an emotional experience (Cacioppo, Berntson, & Klein, 1992). Physiological responses to environmental challenges are controlled by the autonomic nervous system (ANS) which is divided into two subdivisions: the sympathetic one, which prepares the body for engagements such as fight or flight, and the para-sympathetic one, which is responsible for restorative functions such as muscle relaxation. To date empirical evidence focused the explanation on physiological arousal at the very basic level of organismic survival (Campos, 1995; Fischer & Tagney, 1995) and rarely went beyond this scope (Porges, 2001; Tracy & Robbins, 2004). Self-conscious emotions are crucial for psychological function and unlike primary emotions they received considerably less empirical attention.

Methodological roadblocks restrict the study of self-conscious emotions (Lewis, Sullivan, Stanger, & Weiss, 1989). Stimuli and films are less effective in reproducing a self-conscious emotional experience and it is difficult to imagine experimental protocols that are within ethical boundaries (Tracy & Robbins, 2004). Self-conscious emotions compared to primary emotions have an indirect role in adaptive fitness and survival, served through the attainment of social goals by fitting well within a social circle. Primarily self-conscious emotions require self-awareness. To elicit or even feel conscious emotions one needs to be aware of their actions and reflect upon some actual or ideal self-representation. Guilt, shame, embarrassment, sympathy, altruism and pride function within social dynamics coordinating individuals' behaviors leading to acts that are socially valued for the coherence and well being of a social group but also of the self (Tangney & Dearing, 2002; Keltner & Buswell, 1997). Social physiology or the physiology of self-conscious emotions is one of the pillars of normal cognitive, affective and social function since it guides morality within society. Individuals that do not function in response to "appropriate" social cues are socially ostracized and regarded as "strange" since they do not function within social norms (Comer, 2009; Campos, 1995; Fischer & Tagney, 1995).

Mental disorders such as schizophrenia, depression, anxiety, and post-traumatic stress disorders have been associated with prefrontal hypo-activity (Thayer & Friedman, 1997). This dysfunction is directly linked to autonomic de-regulation (Thayer & Brosschot, 2005) that leads to prolonged de-inhibition of the sympathetic-adrenal axis, characterized as allostatic load (McEwen, 1998). Absence of neural inhibition in psychopathology leads to poor affective regulation and processing (Thayer & Friedman 2004). The somatic marker hypotheses, advances the view that bodily arousal is the connecting link between previous events and the feelings associated with the representation of those events (Damasio, 1996; Damasio & Carvlho, 2014). During an external challenge, if bodily arousal is malfunctioning or absent, then the central nervous system that receives inputs from the body will end up associating somatic states with irrelevant memories, which will result in behavioral mismatch (e.g. absence of facial expression, anhedonia). Moreover, if one's embodied cognition is distorted, sympathy for others can be seriously jeopardized (Gallese & Goldman, 1998). Cortical networks that have been suggested to perform the action of CNS-ANS integration in goal directed behaviors and adaptability have been the central autonomic network (Bennaroch, 1993, 1997), the anterior executive region (Devinsky, Morrell, Vogt, 1995) and the emotion circuit (Damasio, 1998). All of the proposed networks of autonomic regulation postulate that bottom up sensory inputs feed information to the prefrontal cortex in order to adapt physiological responses and engage in goal-directed behaviors (Thayer & Friedman, 1997). Failure to do so leads to dysfunction in executive function, social, and affective motivational behavior (Thayer & Brosschot, 2005).

Peripheral physiology monitoring can provide insights to self-regulation in an affective social context (Thayer & Brosschot, 2005). Restoring the body's physiological balance is crucial in restoring healthy mental function and emotional fitness. To date many treatments for personality disorders focus on cortical neurotransmission. However, since there is a loop between the central and the peripheral nervous system called "interoception", it would be wise to focus on more holistic approaches in restoring autonomic function that would eventually lead to healthy mental function and neurochemical restoration. Neuroplasticity has shown its strength in many domains of human nature (Klinke, Kral, Heid, Tillein, & Hartmann, 1999) by restoring sensory input. The body is a large sensory organ, which is not only used to understand one's internal sensory and emotional states but also another's (Gallese, Keysers, Rizolatti, 2004; Rizzolatti, Fogassi, Gallese, 2001; Ebisch, Perucci, Ferretti, Del Gratta, Romani, Gallese, 2008; Wicker, Keyser, Plailly, Royet, Gallese, Rizzolatti, 2003). By treating the body one could potentially treat the mind. After all therapeutic physiological application do exist that show that the effectiveness of addiction treatments relies largely on autonomic restoration (Weise, Krell, Brinkhoff, 1986; Rossy & Thayer, 1998; Reed, Porges, Newlin, 1999; Stein & Kleiger, 1999; Nabors-Oberg et al., 2002; Ingjaldsson et al., 2003).

Peripheral physiology is an important element of human emotional experience and the time is ripe to focus the attention of affective physiology onto self-conscious emotions. Living in a century of technological advancements one cannot ignore the leaps that have been made in the domain of life sciences but rather embrace them along with the plethora of knowledge that has been acquired through gold standard methods (Ioannou, Gallese, Merla, 2014; Berntson, Cacioppo, Quigley, 1993). Infrared thermal imaging gives the opportunity to design experiments that resemble real life situations illustrating how physiology reacts in a social context (Ioannou et al., 2013; Ioannou et al., 2014) and how two individuals physiologically resonate within a social context (Ebisch et al., 2012). Over the past four years methodological difficulties of the fITI have been analysed and a model has been established on the potentialities and limits that the technique has to offer in affective neuroscience (Ioannou, Gallese, Merla, 2014). Thermal imaging provides a novel avenue for the study of social psychophysiology as the face, in addition to its communicative value, provides easy access to contact-free affective physiological monitoring.

The present PhD dissertation focuses on this new approach applied to the study of affective social neuroscience. In the following chapters the potentialities and limits of fITI are illustrated (Chapter 2) and three studies are presented where fITI has been applied to investigate the autonomic signature of guilt in children (Chapter 3); the facial imprints of autonomic contagion in mother and child (Chapter 4); the role of social proximity and gaze in modulating facial temperature (Chapter 5). General conclusions and future directions (Chapter 6) conclude the dissertation.

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Chapter 2

2. Thermal Infrared Imaging in Psychophysiology: Potentialities and Limits

2.1. Introduction to Thermal Imaging

From Hippocrates' early understanding, to Galileo's famous thermoscope and today's technological advancements for remote temperature measurements, scientists have always been fascinated by diagnostic temperature phenomena (Ring, 2004). Sir William Herschel in 1800 became the first scientist that measured heat beyond the visible spectrum (Ring, 2000). Following his father's discovery, John Herschel produced the first "thermogram" in 1840 using sunlight and the evaporograph technique. Current technological advancements allowed measurement of emitted infrared heat by electronic thermal imaging (Ring, 2004; Ring, & Amer, 2012).

Thermal infrared imaging, by harnessing the body's naturally emitted thermal irradiation, enables cutaneous temperature recordings non-invasively, ecologically and contact free. The autonomic nervous system (ANS) is at the forefront of biological heat displays, unconsciously controlling heart rate, breathing, tissue metabolism, perspiration, respiration and cutaneous blood perfusion, providing ground for observations of emotional inference to be made. Thus thermal infrared imaging (also referred to as functional infrared thermal imaging, fITI), enables the characterization of the competing subdivisions of the ANS (as demonstrated by previous research in the field, see Ioannou, et al., 2013). Bioheat-based computations of thermal infrared signs have in their majority been based on individuals' faces. This preference is due to the fact that the face is not obscured and open to social communication and interaction.

Unlike conventional methods of autonomic monitoring, fITI provides versatility. It enables recording of perspiration (Ebisch et al., 2012; Pavlides et al., 2012), cutaneous and subcutaneous temperature variations (Merla, Donato, Rossini, & Romani, 2004; Hahn, Ross, Whitehead, Albrecht, Lefevre, & Perrett, 2012), blood flow (Puri, Olson, Pavlidis, Levine & Starren, 2005), cardiac pulse (Garbey, Sun, Merla, & Pavlidis, 2007) as well as metabolic breathing patterns (Pavlidis, et al., 2007). The reliability of this tool has been repeatedly proven with the use of simultaneous recordings, grounding fITI on golden standard methods, as ECG, piezoelectric thorax stripe for breathing monitoring or nasal thermistors, skin conductance or galvanic skin response (GSR). As for the latter, studies have demonstrated that fITI and GSR have similar detection power (Coli, Fontanella, Ippoliti, Merla, 2007; Kuraoka & Nakamura, 2010; Pavlides et al., 2012; Shastri, Merla, Tsiamyrtzis, & Pavlidis, 2009) (see also figure 4, in Pavlides et al., 2012, p. 6). It has also been suggested that fITI not only enables one to infer psycho-physiological excitement but also to differentiate between baseline and affective states (Nhan & Chau, 2010).

The Autonomic Nature of the Thermal Print of Psychophysiological Responses

Human temperature is of particular significance to medicine. Homeostatic control of cutaneous temperature is functional to both biological and psychological reasons, such as: to face an environmental change, to fight a virus (e.g fever) (Skitzki, Chen, Wang, & Evans, 2007), or to support physiological demands in case of an external threat (Porges, 2001). However, temperature control associated with emotional reactions is far more complex, as it serves a different purpose, has distinct neuro-regulatory systems and carries its own thermal imprints.

Triggered by cognitive appraisal of affectively charged events, the human body deploys physiological strategies that cover any environmental demand, ranging from social interaction to “fight or flight” responses. These responses are controlled by cortical segments that are phylogenetically organised in three evolutionary hierarchical structures (Porges, 2001). The newest division, the frontal lobe, inhibits more primitive lower medullary structures, enabling vagal engagement and fostering among others social interaction. In such occasions, the environment is perceived as safe. Thus the parasympathetic nervous system (PNS) permits normal physiological function that entails non-emergency, vegetative states, (Porges, 2001; 2009). Conversely, in bodily or emotionally threatening circumstances, the 10th cranial nerve, or vagus nerve, is disengaged (Porges, 1992), enabling the sympathetic nervous system (SNS) to take action preparing the body for the fight or flight response. Neural evaluation of threat does not require conscious awareness and involves subcortical limbic structures (Morris, deBonis, & Dolan, 2002) that receive primary inputs from the auditory and visual cortex (Uwano, Nishijo, Ono, & Tamura, 1995).

The amygdala is at the forefront of the defense response, acting as the gatekeeper for the initiation of the SNS. Located in the centre of the cortex, the amygdala affects two main cortical structures. Forebrain areas receive information for the initiation of threat engagement strategies (Lacroix, Spinelli, Heidbreder, & Feldon 2000); specialized neurons in the prefrontal cortex appraise pleasant or unpleasant events in less than 8 milliseconds (Kawasakir et al. 2001). Inputs are also received by the hypothalamus, preparing the body for periods of vigorous physical activity (Owen et al. 2006). To achieve such a goal, the hypothalamus sends nerve impulses to the adrenal medulla for the release of steroid hormones, epinephrine and norepinephrine, enabling alertness and metabolic output (Kallat, 2009). Two biological mechanisms enable thermal effects of affective nature; subcutaneous vasoconstriction and emotional sweating. Activated by epinephrine release in the blood stream, subcutaneous vasoconstriction is a threat response that minimizes the blood volume within veins under the skin (Kistler et al., 1998; Pavlidis, Levine & Baukol, 2001). This mechanism protects against excessive blood loss and possible haemorrhage in case of an injury and is concentrated on the most exposed parts of the body (Chien, 1967; Haddy, Overbeck, Daugherty, 1969; Vatner, 1974; Pearce & Allecy, 1980; Vianna & Carrive, 2005). On the contrary, once threat has been faced, vascular relaxation is observed accompanied by a gradual temperature rise resulting from parasympathetic restoration (Nhan & Chau, 2010). Laser Doppler flowmetry and photoplethysmography suggest that changes in microcirculation caused by subcutaneous vascular constrictions or dilation needs to last for at least 5s for temperature decreases to take place (Kistler et al., 1998). Emotional sweating is activated by norepinephrine binding on sympathetic preganglionic neurons that are situated on the spinal cord. This enables acetylcholine release in the synaptic cleft, which stimulate secretion at the sweat glands. This physiological phenomenon occurs mainly in specific body parts such as the palms, axillae and soles of the feet. This increases elasticity and reduces friction of the skin in regions that have increased contact with the environment and external objects (Porges, 1992; Kamei, et al., 1998; Vetrugno, Liguori, Cortelli, Montagna 2003).

The ANS also regulates facial muscles. Controlled by the brainstem through the myelinated vagus these muscles cause expressions according to psychological and environmental factors (Nhan & Chau, 2010). Constituting an integral part of interpersonal socio-emotional signaling, facial expressions have been argued to be a behavioral gateway for healthy psychological function (Porges 2001). Facial muscles, as all organs of the body, require nutrients supplied through the blood stream.

Thus adjustments in blood flow occur to cover muscular activity changing the emitted thermal print. Periorbital (Levine, Pavlides, Cooper, 2001) and supraorbital vessels (Puri, Olson, Pavlidis, Levine, Starren, 2005) of the face have been observed to show heat escalations according to stressors which are believed to facilitate preparadness for rapid eye movement in flight or flight (Pavlidis, Levine, & Baukol, 2001). During occasions as such, and particularly startles, a temperature dip on the cheeks was observed, likely the result of redirected blood to the eye musculature (Pavlides et al, 2001) as well as of emotional sweating (Merla, 2007). The supraorbital and periorbital vessels feed the main muscles surrounding the eyes; the corrugator, procerus and the orbicularis oculi. The periorbital region has been suggested to carry information about short lived stressors such as startles (Levine, Pavlides, Cooper, 2001; Nakayama, Goto, Kuraoka, Nakamura, 2005) which are controlled by the midbrain central gray, and the nucleus of the tractus solitaris in the pons (Zhao & Davis, 2004). Supraorbital regions have been also postulated to represent prolonged periods of stress due to mental engagement (Zhu, Tsiamyrtzis, Pavlides, 2007; Puri, Olson, Pavlidis, Levine, Starren, 2005). Although the above findings are interesting, literature on the current topic is rather inconsistent, since different researchers although using similar if not identical experimental procedures, found two different sets of results on the effect of startles (Calvin, & Daffy, 2007; Gane, Power, Kushki, & Chau, 2011).

The Validitiy of Infrared Imaging

To examine the reliability of the maxillary area for inferring levels of peripheral arousal Pavlides et al., (2012), the collected temperature values from two infrared cameras were validated on GSR. Temperature measures were taken from the index finger and the upper lip while in total 18 participants underwent a startle task. The synchronicity of the physiological measures was assessed on three parameters a) onset of activation, b) peak, and c) offset (relaxation). In total the experiment lasted 4 minutes in which 3 startles were presented. In all three critical times the GSR showed a strong positive correlation with the temperature of the maxillary area and the index finger (3 x 3 x 2).

Intensity-wise evaluation of the two slopes, ascending (onset-peak) and descending (peak-offset) showed that the signal trend of the GSR in each event is indistinguishable from the thermal print of the two regions of interest (2 x 3 x 2). Despite differences in the onset of the physiological change, Merla and Romani (2007) observed a negative relationship between GSR and the thermal print of the palm and face in 10 participants that received a mild electric shock. In a seminal study Kistler, Mariazouls, & Berlepsch, (1998) using Laser Doppler flowmetry, photoplethysmography and thermal imaging investigated whether changes in fingertip temperature are caused by sympathetic subcutaneous vasoconstriction. By employing a variety of stressful stimuli for the body such as horror movies (Kistler, et al., 1998), cotton swab chewing (Kistler, Mariazouls, & Berlepsch, 1998), acupuncture and deep inspiration (Kistler et al., 1996) it was observed that immediate blood flow changes as a result of vasoconstriction cause the temperature of the dorsal and palmar fingertips to decrease 15 s after stimulation. To establish whether changes of skin temperature were the result of subcutaneous constrictions, the percentage of coincidence was calculated between blood flowmetry and the thermal print while taking into account the 15 s delay. In total 30 individuals and 222 vasoconstrictions were analyzed, indicating that 92.3% occasions of decrease in blood flow lead to a fingertip temperature dip. Moving from human to non-human primates, Kuraoka & Nakamura (2011) postulated that GSR and thermal imaging measures yielded the same results during analyses of variance. The magnitude of change, across conditions, for both fITI and GSR was similar, as video illustration of monkeys vocalizing aggressive calls and screams significantly differed from coos. However, during the last phase of the experiment thermal signals and GSR measures were not in agreement. Whereas GSR did not show any significant differences among visual, auditory and audiovisual presentation of aggressive threats, fITI showed that audiovisual presentations produced a significant decrease in temperature compared with unimodal cognitive cues. Finally, studies on fear conditioned rats showed similar tendencies across physiological measures with fITI. Specifically, exposure of the subject to the fear chamber introduced increases in mean arterial pressure (MAP) by + 26 mmHgm, heart rate (HR) + 80 bpm, body temperature + 1.41 °C, back temperature + 1.2 °C, tail temperature - 5.3 °C, paws - 7.5 °C, eyes + 1.20 °C, and head + 1.5 °C. Changes in MAP where observed within 10 minutes whereas for HR 15 minutes after exposure. Upon return to the home box, levels of arousal returned to baseline levels within 50-60 minutes. On the other hand, thermal imaging of different parts of the body showed a further delay for temperature to reach baseline values. The body took 60-75 minutes, the back 30 minutes, the tail 10 minutes, the paws 15, the eyes 14 and the head 30 minutes to go back to baseline levels. Approaching the reliability of fITI from a behavioral rather than physiological perspective, Ioannou et al., (2013) observed that while temperature of the nose decreased, behavioral signs of distress increased whereas when those signs decreased the nose returned to baseline values. Pavlides et al., (2012) reported that during the laparoscopic drilling task, novices, compared to experts surgeons exhibited substantially more distressful facial signs which were in agreement with higher temperatures as well as activation of perspiration pores on the upper lip. Although the above evidence in animals and humans provide a valuable insight on the reliability of fITI, a direct comparison between physiological measures might not be ideal as they have different response latencies and receive arousal inputs from different control systems.

Creating an Experimental Settting

Cutaneous thermal responses to external stimuli of psychophysiological valence could result in small temperature variations of the regions of interest. Thus it is extremely important to exclude that the observed temperature variations are not artifacts due to either environmental physiological causes or simply to participants' motion. As for the environment, a crucial role is played by the experimental room or set up in which measurements take place. The environmental temperature has to be steady throughout the experimental session. No direct ventilation should hit the subject (Levine et al., 2001). In addition, if possible, room temperature and relative humidity should be set at comfortable values (i.e., thermoneutrality) for the subject. For example, in western countries these values are usually set at approximately 22-24 °C and 50-60%, respectively (Merla & Romani, 2006). Several technical solutions are available in this respect, all of them capitalizing on the continuous monitoring of the environmental conditions (Gane, Power, Kushki, Chau, 2011; Nakanishi & Matsumura, 2008). Other issues that should be taken into account during the experimental setup is the prevalence or absence of systematic sources of thermal noise. These include thermal reflective walls, furniture material, direct sunlight through windows, heat emitting monitors in close proximity to the participant's face. These will result in overestimation or underestimation of the physiological temperature change in given regions of interest. Prior to the initiation of the study, the participant or subject (along with the experimenter) should be left to acclimatize in the room for 10-20 min. This enables the restoration of cardiovascular and respiratory activity, as well as allowing skin temperature to reach a thermal equilibrium with the experimental room (Ring & Ammer, 2006). Some authors suggest prior to the experimental session to remove corrective eyewear because glass is opaque to infrared light. Once removed, give enough time to allow pressure related temperature restoration of the surrounding tissue of the nose (Gane et al., 2011). During recordings, the distance between the camera and the subject depends on the size of the regions of interest to be imaged and the camera's optics. Mostly the camera has been placed 1-3 meters away from the participant (Nakanishi & Matsumura, 2008; Shastri, Merla, Tsiamyrtzis, Pavlides, 2009) or 30-70 cm away from the subject (Kuraoka & Nakamura, 2011; Vianna & Carrive 2005).

Exclusion criteria for participation in a fITI study include aspects related to normal cutaneous thermoregulation; such as peripheral neuropathy, micro and macroangiopathies, connective tissue diseases and psychophysiological disorders. Requirements for participation include abstinence from intaking or consuming vasoactive substances (nicotine, caffeine, alcohol) for at least 2-3 hours prior to participation in order to improve reliability of the assessments (Merla, & Romani, 2006).

Finally, it is important to take into account the circardian rhythm of the human body when conducting an experiment. Recordings should take place for the majority of participants at the same time and season in order to have consistent group comparisons and to be able to observe temperature variations on the same scale. It has been illustrated that skin temperature varies throughout the day. Whereas in the evening the core body temperature and proximal skin temperature rise in contrast to distal skin temperature, the opposite effect seems to take place in the morning (Kräuchi & Wirz-Justice, 1994). Furthermore, since heat exchange with the environment occurs by ''means of conduction, convection, radiation and evaporation'' (Kräuchi, p. 148), different homeostatic mechanisms take place during different seasons. Sweating and increased blood flow followed by peripheral blood vessel constriction is observed in warm seasons whereas the opposite happens in cold climatic conditions. These phenomena occur mainly through smooth muscles in arterioles and arteriovenous anastomoses in distal skin regions such as the fingers, nose, toes and ears (Hales, 1985).

The Selection of an Appropriate Baseline in fITI

Facial thermal prints provide a great channel for inferring emotional arousal. In order to obtain observations of affective nature, the selection of an appropriate baseline represents a major methodological challenge (Levenson, 1988). Establishing an autonomic starting point will be the foundation for the definition of the directionality of the physiological change during emotional arousal. So, for example, if a particular emotion, such as fear, is assumed to cause an increase in supraorbital and periorbital temperature, then the question would be: 'What would make an appropriate baseline?'

The use of 'rest' as a baseline condition provides ground for experimental comparisons. In such occasion, participants are required to 'rest and empty their minds of all thoughts, feelings and memories' (Levenson 1988, p. 24). Using 'rest' as a baseline does give adequate contrast power since during this emotional state the parasympathetic nervous system is active and the vagal nerve is engaged. However, it rarely represents a naturalistic emotional setting. Emotions occur while the ANS and the organism are engaged. Thus it would be better to employ a method that has moderate levels of ANS activation. Opposing or near opposing emotions to the experimental condition could also serve as appropriate baselines. So if happiness is the emotion of interest, then appropriate baselines would be sadness, anger and even fear. This comparison is based on the assumption that the ANS runs on two interlinked opposing subdivisions. Happiness, although a positive emotion, is characterized by an increase in heart rate as a result of vagal withdrawal; however, it differs from negative emotions in terms of peripheral vasodilation (Krebig, 2010, pp. 23). One primary component of temperature change is the change of blood flow to the surface of the skin. Thus if the above emotions are matched consistently in the scientific literature, if not in all, at least in most aspects of physiological activity, peripheral constriction or vasodilation is going to provide the basis on which the thermal observation will be observed. Thus the above mentioned emotions provide a good contrast pair since the ANS is always active and not under the conscious control of the individual (Winkielman & Berridge, 2003; 2004).

The alternative baseline is to eliminate it completely, without examining changes from a reference point (Levenson, 1988). This approach could be used both for between-subjects comparisons and within-subject comparison. Although this method appears to provide an easy solution for defining an appropriate baseline, it has two main drawbacks. No baseline means no directionality description for the target emotion. Second, if an individual is subjected to two different emotional conditions and does not provide measurable autonomic signs in one of the two, then, no matter how good the results of the other condition are, no contrast between the two emotions can be performed.

The average temperatures which has been documented in humans during ‘rest’ or pre-stimulation was 32.3 °C for the palm, 34.75 °C for the face (Merla & Romani, 2007), 30-34 °C for the fingertips (Kistler et al., 1998), 34 °C for the forehead, and 31 °C for the nose (Calvin & Daffy, 2007). Moreover, in experiments in which stimuli were used to establish a baseline, the average temperature for the nose was 33.08 °C (Ioannou et al., 2013), and 35.6 °C for the face (Hahn et al., 2012). In addition, Nakayama et al., (2005) documented during pre-stimulation in four different subjects temperatures of 28.2 ± 0.5, 27.0 ± 0.7, 34.6 ± 0.3, and 33.4 ± 0.3 °C, respectively. Kuraoka & Nakamura (2011) observed an average temperature of the nose during baseline of approximately 34-36 °C. Finally, in the case of rats during rest the body temperature was 37.45 °C, for the back 31.7 °C, for the tail 31.6 °C, paws 34.8 °C, eyes 35.2 °C, and head 32.8 °C.

No gold standard exists for choosing an appropriate baseline. However, different types of baselines have been used across thermal imaging studies and in certain cases they could be easily applied in studies with similar design. Kreibig (2010) provides a review of autonomic reactions during positive and negative emotional states, which should be used as a guide for choosing an appropriate reference point.

Differentiating Emotions

There are two main theories of the autonomic function in emotions: the differentiated (Alexander, 1950) and the undifferentiated ANS (Mandler, 1975; Schacter & Singer, 1962). Whereas the differentiated theory talks about different emotions in multiple patterns of ANS activation, the undifferentiated theory states that all emotions are governed by two ANS subdivisions (Cannon, 1929). In contemporary research, the undifferentiated theory has been the most dominant one. Very few emotions in the arena of the ANS can be specified by their physiological mark, since the ANS is divided in PNS and SNS. However, if multiple physiological measures (e.g. breathing, galvanic skin response, heart rate, blood volume, cutaneous thermal variation) are taken into account (Kreibig, 2010) along with the speed of onset, intensity and duration, then it is possible that specific patterns could be defined for each specific emotion (Levenson, 1988). In support to this argument Nummenmaa, Gleran, Hari, & Hietanen (2013) using a unique topographical self-report method have observed in a large population sample (n=701) that different emotions have statistically separable bodily sensation maps. The collected responses showed that different emotions not only are consciously felt but also represent topographically distinct somatosensory experiences. Finally, although it is assumed that parasympathetic arousal is associated with pleasurable emotions and sympathetic with negative ones, this is not always true. For example lacrimal glands responsible for tear secretion are reached only by parasympathetic efferent nerves (Lutz, 1999). Even positive emotions, such as laughter, exhibit a sympathetic element rather than a parasympathetic one (Nakanishi, Imai-Matsumura, 2008).

Regions of Interest for Studying Psychophysiology with fITI

fITI has been adopted in a variety of studies involving human emotions as well as reflexes. Particularly, it has been used to study startle response, empathy, guilt, embarrassment, sexual arousal, stress, fear, anxiety, pain and joy. To extract information of affective nature, regions of interest are used (ROI). Regions on which most observations in the literature were based upon are the nose or nose tip, the periorbital and supraorbital vessels of the face, usually associated with the corrugator muscle, forehead, and the orbicularis oculi (surrounding the eyes) as well as the maxillary area or the upper lip (perinasal). Regions on which fewer observations were gathered were the cheeks, carotid, eye, fingers, as well as the lips (Figure 1). According to the subjects' response to the emotional stimulus, as well as the region of interest, temperature elevates or decreases. Table 1 provides a summary of the emotions as well as the regions in which observations were based. The direction of the average temperature change in those regions is reported as well (see also Table A1).

Infrared Data Acquisition

To perform thermal imaging recordings researchers have used camera models of different resolution. The majority of studies used a camera with a resolution of 256 x 256

(Merla & Romani, 2007) to 320 x 240 (Calvin & Daffy, 2007; Hahn et al., 2012; Puri et al., 2005; Kang et al., 2006; Kuraoka & Nakamura, 2011; Vianna & Carrive, 2005; Pavlidis et al., 2001; Ebisch et al., 2011; Ioannou et al., 2011; Pavlidis et al., 2012; Shastri et al., 2012; Pavlidis et al., 2002; Zhu et al., 2008). Recordings of higher image quality such as 640 x 480 (Gane et al., 2011; Manini et al., 2013) to 640 x 512 (Pavlidis et al., 2012; Shastri et al., 2012) were, however, relatively more rare. One reason for this is because thermal cameras are rather expensive and their price depends on the quality of the produced image as well as the frame rate. The use of higher or lower image quality is relevant to the experimental question as activation of perspiration pores is difficult to identify at low resolution (Pavlidis at al., 2012). In addition, higher resolution means maximum storage size and when the temporal latency of the thermal print is a variable of interest, then researcher need to sacrifice image quality for higher frame rate (Merla & Romani, 2007; Puri et al., 2005).

In most experiments temperature measurements where extracted continuously. Frame acquisition rate varied from 60 frames per second (fps) (Tsiamyrtzis et al., 2006), 50 fps (Merla & Romani, 2007), 31 fps (Puri et al., 2005; Pavlidis et al., 2012; Shastri et al., 2012; Pavlidis et al., 2002; Zhu et al., 2008), 15 fps (Gane et al., 2011), 1 fps (Ebisch et al., 2011; Kang et al., 2006; Ioannou et al., 2013; Kuraoka & Nakamura, 2011; Manini et al., 2013; Ioannou et al., 2013; Nakanishi & Imai-Matsumura, 2008), 1 frame every 10 s (Kistler et al., 1998; Nakayam et al., 2005) and 1 frame every 75 seconds (Hahn et al., 2012). Other studies have used isolated images equidistant in time to make their analyses, such as Vianna & Carrive (2005) who acquired frames every 2 minutes. In the case of Calvin & Daffy (2007) temperature was documented in isolated time intervals, prior (baseline) and just immediately after the completion of the task, leaving a "physiological washout" period between the experimental conditions. Nevertheless, more frames throughout an experimental task enable the possibility of more efficient movement tracking (Tsiamyrtzis et al., 2006; Pavlidis et al., 2012; Shastri et al., 2012; Pavlidis et al., 2002; Zhu et al., 2008; Manini et al., 2013), yield more temperature data, allow investigation of the temporal occurrence of the physiological phenomenon in more detail and provide the flexibility for discarding frames that are judged unusable because of the participants' movements (Ebisch et al., 2012; Nakanishi & Imai-Matsumura, 2008; Ioannou et al., 2013).

Temperature Extraction

Moving from problems of physiological definition to methodological considerations, ROI size across thermal frames poses a challenge. When it comes to extracting data from single frames, manual fluctuations in the size of ROIs can cause thermal observations of non-physiologic nature, which can lead to false conclusions as a result of methodological inconsistencies. This can be easily tackled, however it needs to acknowledged by any thermographer. Analyses of ecological experiments where participants are let free to move without any restriction are rather time-consuming as frame-by-frame pre-processing is required. All frames need be extracted from approximately the same angle so that temperature artifacts are avoided (Ioannou et al., 2013; Ebisch et al., 2012). Even in this case though frames might appear to have some small temperature fluctuations from ±0.1 to ± 0.2. This is insignificant and will not affect the overall value of the condition. Once all data is extracted, however it is advised that a stem and leaf plot is created to examine for any outliers that might exist in the data set so that they can be eliminated. Most fITI studies have not controlled for the reliability of data extraction. This is a crucial part and should not be underestimated in future studies. To control for reliability issues a rater naïve to the experimental protocol can perform the analyses or the data set can be split in two with two independent raters performing temperature extraction, since manual extraction of temperature is quite laborious. Nevertheless, probably the best possible solution in which complete confidence can be provided to data extraction is if the leading scientist performs the initial pre-processing of frames for all participants and then provides the selected frames for inter-rater evaluation. The second rater will then select and evaluate a sample of the participants without the knowledge of the primary rater for at least 1/3 of the sample. Then the mean for each of the participants will be calculated for each condition and compared between the two raters using analyses of variance to examine if significant differences between the two data sets exist. Inter-rater reliability can also be assessed using a Kappa Measure of Agreement, however the tendency of the temperature from baseline to experimental condition will first need to be assessed for each individual using a Mann-Whitney U and then coded categorically for each condition as a) ascending, b) descending, or c) stable. In addition, probably the best solution would be to calculate the mean degree of change from one condition to the other for each individual and then run a Kappa Measure of Agreement or an Analyses of Variance to examine if inter-rater coding is in agreement. Studies with automatic tracking on the other hand do not entail dangers as such (Manini et al., 2013; Tsiamyrtzis et al., 2006; Zu et al., 2007). Particularly Dowdal, Pavlidis, & Tsiamyrtzis (2006) have illustrated a tracking method using coalition game theory, which accurately monitors the motion of the targets surface even if the surface is partially occluded or uneven. The main principle of this technique lies on the fact that regions of interest or trackers "communicate" with each other as they are interlinked in a shape divided by smaller surface areas. In situations in which a particular region of interest is occluded because of the participant's movement, the rest of the trackers that are still on target continue to reliably track tissue by "tipping off" others that have gone off view. This computational method can automatically extract IR data from regions of interest (e.g. nose), continuously, without losing consistency across measures or frames, and without introducing noise due to the participants' motion or due to human error.

Finally, manual data extraction compared with automated tracking gives the researcher the compete control over the data set as even in the occasion of tracking, if unnoticed, movement can still induce some noise artifacts (Gane et al., 2011). Although the tracking algorithm by Tsiamyrtzis et al., (2006) probably provides the best possible solution in terms of software tracking in either voluntary or involuntary movement, Gane et al., (2011) suggest that "in an access context, multiple thermal imaging devices positioned at different angles may be required to ensure that the user's face remains in view" (pp. 7).

Statistical Testing of Heat Maps

The analyses of the collected heat prints across conditions has so far been based on the research question as well as the way in which physiological arousal was collected and coded. The majority of experiments have extracted temperature based on the average heat signature of pixels in a region of interest (Ioannou et al., 2013). However, Kuraoka and Nakamura (2011) as well as Nakayama et al. (2005) measured temperature on a region of interest, pixel by pixel and reported the absolute temperature of single pixels. This was possible only because the subjects were relatively static and corrections were still applied for movement artifacts. In general studies have used analyses of variance or their non-parametric alternative to draw conclusions about differences between conditions and groups. Prior of any type of analyses the mean temperature for each ROI and individual was calculated and the mean of each phase was compared using one way repeated measures analyses of variance (ANOVA) (Kang et al., 2006; Gane et al., 2011; Ioannou et al., 2013; Ebisch et al., 2012; Manini et al., 2013; Vianna & Carrive, 2005; Merla & Romani, 2007; Kuraoka & Nakamura, 2011). In the non-parametric alternative of repeated measures ANOVA, a Friedman test was used by Nakayama et al., (2005) to evaluate changes on nasal skin temperature during different respiration rates. In situations, however, where measurements took place for separate groups one-way between groups ANOVA (Kuraoka & Nakamura, 2011) with a Tukey HSD, a two-way between groups ANOVA (Pavlidis et al., 2012) with a Tukey HSD or a Kruskal-Wallis test (Nakanishi & Imai-Matsumura, 2008) followed by a Steel-Dwass test have been used. Moreover, when temperature was assessed for less than three conditions, the majority of experiments used a paired sample t-tests with Bonferonni corrections (Pavlidis et al., 2012; Kuraoka & Nakamura, 2011; Kistler et al., 1998; Hahn et al., 2012; Nakayama et al., 2005) or a non-parametric Wilcoxon Matched Pair Signed Ranks (Calvin & Daffy, 2007). Others have used algorithmic computational application to assess intra-individual changes by first segmenting periorbital and supraorbital blood vessels and then calculating changes in heat patterns between conditions (Tsiamyrtzis et al., 2006; Shastri et al., 2012; Pavlidis et al., 2002; Zhu et al., 2008; Gane et al., 2011). At the individual level only two studies have examined temperature changes across conditions and they used a paired sample t-test (Nakayama et al, 2005) or a Mann-Whitney U (Ioannou et al., 2013).

Temporal Latency of Cutaneous Temperature Change

Thermal signal development as a result of vascular change, perspiration, or muscular activity is rather sluggish compared with other measures of physiological arousal (Kang, et al., 2006). Kuraoka & Nakamura (2011) observed that galvanic skin response (GSR) has a latency of 3s whereas the fastest observable change that can be recorded with thermal imaging is 10s. In addition Merla and Romani (2007) reported the same GSR latency compared with a thermal response of 3.8 s after stimulus onset. To investigate the timing of the temperature change, repeated measures ANOVA with Dunnett's t-test (Ebisch et al., 2011; Kuraoka & Nakamura, 2011) and paired sample t-tests with Bonferonni correction have been used (Kuraoka & Nakamura, 2011; Nakayama et al., 2005; Kistler et al., 1998). To examine changes in temperature using t-tests researchers selected frames equidistant in time from the pre-stimulation and post stimulation periods. All studies prior of any analyses have calculated the average value of each frame and reported their results at the group level. Only Nakayama et al. (2005) provided statistical changes in temperature that occurred also at the individual level. In the case of Kuraoka & Nakamura (2011) a total of 10 frames were extracted from each individual for the t-test. Three of those (30 s, 20 s, 10 s) were prior of the stimulation (0 s) and six after (10 s, 20 s, 30 s, 40 s, 50 s, 60 s) the stimulation. For Dunnett's t-test eight frames in total were selected, taken 10 s prior of the stimulation, during stimulation 0s and after stimulation with a 10 s time difference (10 s, 20 s, 30 s, 40 s, 50 s, 60 s). During these test results have shown that fear induction significantly decreases the temperature of the nose of the monkey, on average within 50 s (Nakayama et al., 2005). At the individual level these changes have taken place from 10-110 s, lasted 220 s-280 s during and even after stimulation. Pixels of the nose, which had shorter response latencies (10 s), were situated on the bridge of the nose whereas pixels with delayed responses were located on the nasal tip. Another study in the fear domain observed on average changes within 20 s of stimulus presentation that lasted in excess of 60 s (Kuraoka & Nakamura, 2011). Kistler et al. (1998) by using thermal infrared imaging and laser Doppler flux provided a valuable insight into the delayed response of the thermal print. The researchers while controlling for perspiration patterns with GSR, concluded that cutaneous temperature change occurs only if decreases in subcutaneous blood flow are present for at least 5 s, leading to a heat evasion of 15 s after stimulation, averaging a delay of approximately 20 s. Although the above observation was made on dorsal fingertips, the same phenomenon is true for facial skin temperature as the layer of flesh is very thin (Pavlidis, et al., 2001). From human to mice the latency of the thermal print looks different. Fear conditioned mice showed that different parts of the body have different response latencies. Re-exposure of the subject to the aversive shock box caused an increase in body temperature. When the rat was returned to the home box, body temperature took 60-75 minutes to return to baseline values. The same temperature tendency was observed also for the back and the head of the animal, with temperature values descending to baseline 30 minutes after exposure. The eyes of the animal also rose in temperature but unlike the above regions baseline temperatures were reached in 14 minutes. On the contrary, the tail and paws showed a decrease in temperature, after re-exposure to the shock box. Once the animal was in a safe environment again, temperature started to rise reaching baseline levels within 10 minutes for the tail and 15 minutes for the paws. In a similar experimental context Merla & Romani (2007) observed that sub-painful stimulation caused a decrease in temperature of the face at 3.8 s that lasted for approximately 15-20 s on average after a mild electric shock was delivered. On the other hand, reports on startles by Pavlides et al. (2001) document a temperature change in less than 30 s upon stimulation. Moving away from fear and into the context of guilt and empathy, Ebisch et al. (2011) observed temperature changes at the maxillary area and nasal tip, 10 s after each condition and lasting approximately 20-30 s regardless of distress or soothing. Finally, Nakanishi & Matsumura (2008) observed that 2-3 months old infants had a decrease in nasal temperature 2 minutes after the onset of laughter whereas in 4-6 months old and 8-10 months old temperature had significantly decreased within the first 15 s. All three infant groups showed the same temporal dip on the forehead and cheeks 2 minutes after the onset of laughter.

2.2. Empirical findings of emotions studied with fITI.

Stress

Working conditions are governed by mental tasks. Non-intrusive physiological measures for assessing mental workload are required and fITI may provide a possible solution. Focused on professional drivers, a study of occupational ergonomics assessed mental workload using fITI. Participants were exposed to simulator driving tasks both in the city as well as on the highway while cognitively challenged with a Mental Loading Task (MLT). Compared with temperatures of the pre-driving session (baseline), significant differences were observed on the nose temperature across all conditions. The MLT seemed to have a defining effect on the temperature decrease of the nose, which dropped 0.55 °C below baseline during the simulated city drive. No significant changes were observed on the forehead (Calvin, Duffy 2007). Unlike other surfaces of the body, the forehead has the most stable temperature (Stoll, 1984). Staying in the occupational arena, in a seminal study, levels of stress in expert and novice surgeons were measured during training on 3 different drilling tasks, designed for laparoscopic surgery. The authors monitored the peri-nasal facial regions of participants and observed higher levels of distress in novice compared with expert surgeons. Distress signs were assessed by lower temperatures of the peri-nasal region along with the activation of perspiration pores (Pavlidis, Tsiamyrtzis, Shastri, Wesley, Zhou, Lindner, Buddharaju, Joseph, Mandapati, Dunkin, Bas, 2012; Shastri Papadakis, Tsiamyrtzis, Bass, Pavlidis, 2012) (see also figure 2 in Pavlides et al., 2012, p. 4). In another study, focused on the topic of human-computer interaction, authors used a stroop task to exploit wirelessly signs of frustration. Based on frontal forehead regions they observed that compared with 'rest', stress increased blood volume into supraorbital vessels, which in turn dissipated convective heat (Puri, Olson, Pavlidis, Levine, Starren, 2005; Zhu, Tsiamyrtzis, Pavlidis, 2008). Thermal IR imaging has also been used to assess affective training times. Learning proficiency patterns were based on an alphabet arithmetic task (Kang, McGinley, McFayen, Babski-Reeves, 2006). During the first trials, nose temperatures were lower as a sign of task difficulty. However, with increased task proficiency after training trials, nose temperatures rose systematically across the 7 blocks as individuals became more accurate and quicker in their responses. Forehead temperature remained the same throughout the experimental phases. Moving from affective learning to child development Mizukami, Kobayashi, Ishii, Iwata (1990) studied early infant attachment with the help of thermography. Infants were exposed to 3 different experimental phases including separation from the mother, a short-lived replacement of the mother by a stranger, as well as the infant being in the presence of the mother and a stranger. By observing negative temperature changes on the infants' forehead, the researchers illustrated that infants are aware of strangers and that infants form an attachment earlier than previously thought, specifically from 2-4 months after birth.

Fear

In an interesting experimental paradigm, Kistler, Mariauzouls and Berlepsch (1998) induced fear in participants through scenes from a thriller movie. The shower scene of Alfred Hitchcock's movie 'Psycho' was used to induce fear. Baseline comparisons prior of the killing section showed that temperature changes of the fingertips reached decreases of up to 2°C as a result of vasoconstriction. In another study, Merla & Romani (2007) studied thermal signals of the face in fear-conditioned individuals. Unexpected sub-painful mild electric stimuli were randomly delivered to the subject's median nerve. Results showed a reduction of temperature and sweating on the perioral region, forehead and palms. Despite the above human studies, the majority of fear related studies involve animals. Monkeys exposed to a video of a raging monkey along with monkey aggressive threats had the most marked temperature decrease on the nose than any of the above stimuli presented alone (Kuraoka & Nakamura, 2011). On the other hand, sounds produced in response to food and as a result of separation from the mother or the social group produced no significant changes on the nasal temperature in comparison to baseline (Kuraoka & Nakamura, 2011). These authors concluded that the temperature of the nose should be used as an indicator of affective states in animals. In another experiment involving monkeys, fear was elicited with the approach of a threatening person. Temperature decreases were documented on the nasal regions, whereas temperature increases on the eyelid's adjacent regions as well as the region under the nostrils were considered inconsistent among subjects (Nakayam, Goto, Kuraoka, Nakamura, 2005). Rats have so far been the subjects of preference for the majority of neuroscience laboratories. In an interesting study, fear-conditioned rats showed temperature decreases in their paws and tails as a response to the shock box (designed to deliver small electric shocks). On the contrary, subjects' return to the home box restored body heat to baseline temperatures (Vianna, Carrive, 2005).

Startle

The startle response is a natural reflex. In real life situations, it occurs when individuals are cognitively engaged with a task and a sudden event requires immediate shift of attention followed by rapid somatic (i.e., motor and behavioral) responses such as blinking or avoidance. Shastri, Merla, Tsiamyrtzis, and Pavlides (2009) managed to grasp this element of surprise by using natural sounds (such as glass breaking and phone rings) as the experimental condition. Baseline recordings were made while individuals were performing a mental task (counting randomly appearing circles on the monitor). Results showed that during startles, perspiration pores on the maxillary area became active, decreasing the temperature of the upper lip and surrounding regions (see also figure 1 in Shastri et al., 2012, p. 367). In addition, temperature increases were observed on supraorbital and peri-orbital regions of the face. Naemura, Tsuda & Suzuki (1993) observed that compared with a startle of 45 dB, white noise of 100 db causes nasal skin temperature to decrease. Pavlidis, Levine, & Baukol, (2001) reported similar results in a study in which participants were exposed to a loud startle of 60 dB after sitting quietly in a dark room. Temperature increases were observed on the peri-orbital and neck areas (over the carotid) whereas participants' cheeks cooled down. In order to better understand this physiological phenomenon of heat distribution, researchers injected epinephrine (adrenaline) shots to the participants' hand. Evacuation of heat from the injection point to the surrounding vasculature and tissue was observed. Taking into account the warming of the carotid, investigators accounted the selective heat change of the face to the adrenergic system, further suggesting the redirection of blood from the cheeks to the peri-orbital region. On the other hand, similar experimental protocols did not yield the same results and investigators reported no temperature changes on the peri-orbital regions as a response to startle (Gane, Power, Kushki, Chau, 2011). In this experiment, participants were required to complete an image-matching task and they had to press a button when image pairs matched. At unexpected time intervals, the 102 dB auditory startle stimulus was presented.

Empathy

Studies involving children have used a more controlled and well-thought baseline than ordinary studies. When empathy was studied between children and their mothers in distressful situations, the investigators (Ebisch et al., 2012) made sure that the child would first feel safe and comfortable with the experimenter. This was achieved initially in the presence of the child's caretaker, when the experimenter started neutrally interacting with the child, allowing enough time for the child to psycho-emotionally familiarize with the stranger. Then baseline recordings were taken in the absence of the mother, while the experimenter continued playing with the child. The mother was able to observe the child behind mirror glass. In order to induce distress and measure the thermal facial synchrony of the mother and the child, a toy, which was preplanned to break in the child's hands, was used as a stressor. Unaware of the situation, both participants showed simultaneous temperature decreases on the maxillary and nasal region of the face. Once the mishap was restored during the soothing phase of the experimental session, both maxillary and nasal region increased in heat (see also figure 1 in Ebisch et al., 2012, p. 4). An extension of the above study including an additional group of female participants showed that mother--child dyads in contrast to other-women--child dyads have faster empathic reactions to the child's emotional state (Manini et al., 2013).

Guilt

The above mentioned studies used an experimental paradigm inducing guilt, further explained in Ioannou et al. (2013) and in Chapter 3 of the present dissertation, with a particular focus on the sympathetic relevance of the nose tip for each individual child. Alhtough the group analyses in the above study yielded a relatively clean effect for the mishap and soothing phase, at the individual level physiological responses throughout conditions were not always in the same direction. Significantly different responses were observed in 8 out of 15 children during playing, with 2 showing an increase and 6 a decrease of temperature. Mishap on the other hand was much more homogeneous with 12 out of 15 children showing a dip in temperature. The entrance of the experimenter was not marked with the same group tendency as 4 out of 15 children reached a signifficant temperature decrease and 7 an increase. Finally during the soothing phase 12 children showed a rise in temperature compared with previous conditions (see also figure 1 in Ioannou et al., 2013, p. 6)

Interpersonal contact and sexual arousal

Sexual arousal has been characterized by sympathetic influences. To establish a direction of the thermal print while participants viewed an erotic movie for 5 minutes, researchers used a sport movie as a reference point for temperature comparison (Merla & Romani, 2007). During stimulation the temperature of the forehead, lips and peri-orbital regions increased. In another study Hahn, Whitehead, Albrecht, Lefevre and Perret (2012) used interpersonal physical contact through a handheld light-flashing device to examine social contact and sexual arousal. As a baseline an acclimatization period was used in which participants viewed a series of emotionally neutral faces. Physical contact through the handheld device was performed on different parts of the body such as the face, chest (high-intimate), arm and palm (low intimate) from both male and females experimenters It was observed that the temperature of the face increased. Specifically, it was observed that when high-intimate regions were touched, temperature increase was higher, reaching even higher levels when that act was performed by the opposite sex. This temperature increase was mainly localized on the mouth, nose, and the peri-orbital regions of the face.

Embarrassment

Providing the only study for embarrassment, Merla & Romani (2007) exposed participants to perform a stroop task in front of unknown people. The study was designed in order to elicit feeling of embarrassment and mild stress when the participants performed the task wrongly in the presence of others. The pre-stimulation period was used as the baseline. Temperature decreases were observed on the palm, the face, and especially around the mouth, due to emotional sweating. Thermal data were grounded and validated on GSR recordings.

Joyful expressions

Laughter is a crucial part of social interaction and it carries its own autonomic print. Researchers from Japan studied joyful expressions in infants across three age groups (2-3, 4-6, 8-10 months old) in which laughter was elicited during playing with a stranger. For baseline measures the pre-stimulation period was used, prior to the onset of laughter (Nakanishi & Matsumura, 2008). All age groups showed a temperature decrease on the nose compared with the forehead and cheeks that remained rather stable. This change was more evident in 4-10 month old infants. The nose temperatures of the two older age groups (4-6, 8-10 month old) significantly differed from 2-3 month old infants. The nose temperature of a 4-6 months old infant started increasing 15-30 s after laughter onset but then decreased after 1 minute. In addition, five out of ten 4-6 months old had a decrease greater than 0.5 °C after 1 minute and among those five, three children displayed a decrease greater than 1 °C. Five out of six 8-10 month old infants had decreases in nose temperature within 15 s. On the forehead temperature change across individuals was more homogeneous as all age groups showed decreases of less than 0.5 °C, 2 minutes after laughter onset. On average the forehead dropped for 2-3 month old infants 0. 5 °C whereas for the 4-6 and 8-10 infant group it was 0.1 ± 0.33 ◦C for the former and 0.3±0.24◦C for the latter. Temperature on the cheeks of 2-3 month old was less than 0.5 °C, 2 minutes after laughter onset, whereas in the older age groups large individual variability was observed. While an infant of 4-6 months old showed an increase of more than 0.5 °C another showed a decrease of 1 °C. `Overall in the 4-6 age group there was a 0.2 ± 0.40 °C decrease after 2 minutes. Finally the cheek temperature of three out of six 8-10 month old infants period showed a change of less that 0.5 °C during the 2 minute period. All 8-10 month old infants had an average increase of 0.1 ± 0.45 °C after 2 minutes.

Lie Detection & Deception - Anxiety

Due to the strong nature of fITI in reading emotional states, a variety of studies have employed this technique in the context of lie detection for home security purposes. Pavlidis, Eberhardt & Levine (2002), in order to assess the reliability of fITI in identifying thermal patterns of deceit, devised an experiment in which participants had to stab a dummy, steal 20 $ and then assert their innocence about the crime. The pre-stimulation period was used as the baseline and temperature was measured prior of the participants claiming their innocence to the critical question “Did you steel the 20 $”. Thermal imaging managed to accurately identify 11 out of 12 subjects as guilty through the increase of cutaneous perfusion in the peri-orbital region, while temperature increases were observed on the forehead and in regions surrounding the eyes. Following the same experimental approach, with additional number of participants, other studies on the topic focused at particular regions of the face. Tsiamyrtzis, Dowdall, Shastri, Pavlidis, Frank, Ekman (2006) suggested that temperature monitoring of the peri-orbital vessel during interrogation provides 87.2% accuracy in detecting deceptive individuals. In addition Zhu, Tsiamyrtzis, & Pavlidis (2008), by focusing on the forehead and particularly on the corrugator muscle supplied by supraorbital vessels, gave 76.3% accuracy for lie detection. Temperature increases for the above studies were accounted to increase blood perfusion to facial muscles as a result of mental stress and fight-or-flight response.

2.3. Discussion on the use of fITI in psychophysiology

Benefits

Kistler, et al., (1997) postulates that “acquiring temperature measurements in psychophysiological studies offers the advantage of simplicity of performance and analyses, compared with more complex analyses of flux and pulse volume“ (1997, p. 35). Over the years the sensitivity and resolution of thermal cameras has dramatically improved (Vianna & Carrive, 2005). Physiological events that would otherwise be invisible to the naked eye, such as activation of perspiration pores, can now be observed and documented (Pavlidis et al., 2012). Infrared cameras allow reliable, wireless recordings to take place, from a distance, without interfering with the subject’s behavior (Anbar, 2002; Kastberger & Stachl, 2003; Head & Elliott, 2002; Vianna & Carrive, 2005), or the experimental procedure (Pavlidis et al., 2012). In addition, fITI allows recordings in an ecological experimental setting despite participants' movements (Ebisch et al., 2012; Ioannou et al., 2013; Nakanishi & Matsmura, 2008; Manini et al., 2013). Furthermore, whereas GSR electrolytes can change their conductivity over time (Levenson, 1988), occur spontaneously during baseline recordings (Laine, Spitler, Mosher, Gothard 2009) or during arm movements towards the rewarded stimuli (Amiez, Procyk, Honoré, Sequeira, Joseph 2003), fITI provides the same physiological recording efficiency, accurately reflecting the autonomic nature of the psychological phenomenon throughout the experimental procedure. Moreove,r GSRs are so sensitive to stimuli of emotional value reaching maximum levels of activity in conditions of variable degrees of intensity, making them indistinguishable from each other, an obstacle that fITI overcomes (Kuraoka & Nakamura, 2011). Kreibig (2010) stated that: 'Investigations of ANS responding in emotion have long been impeded by the exclusive use of "convenience measures," such as HR and electro dermal activity, as sole indicators of the activation state of the organism' (as cited in Kreibig, 2010, Measures of activation components, pp. 29). Thermal imaging provides a new tool for psychophysiological monitoring especially valuable in individuals that cannot express their emotional state verbally such as infants (Mizukami et al., 1990; Nakanisi & Matsumura, 2007) and for individual that cannot give subjective measures due to their physical state such as paralyzed patients, patients with locked in syndrome or patients in a coma. Given the right experimental paradigm, fITI can provide novel avenues in the arena of biofeedback, clinical diagnostics, psychopharmacological assessment as well as the efficacy of psychotherapeutic treatments in several types of developmental, social, personality and psychotic disorders.

Limitations

Despite the advantages offered by fITI, thermal signal development as a result of vascular change, perspiration, or muscular activity is rather slow. Kuraoka & Nakamura (2011) stress the fact that fITI has a longer latency than SCR. Specifically, the earliest temperature change can be observed within 10 s compared with SCR where signal changes are evident within 3 s. In addition, most observation with fITI have been based on the nose, however controversy exists as to what might be causing the observed temperature change. Studies that addressed the phenomenon of nasal temperature decrease observed that even when respiration rate changed to two-fold as a result of heavy breathing, no temperature change was observed on subjects' nose (Nakayama et al., 2005). On the contrary, Pavlidis et al. (2001) observed that noses of participants during leisure walking got colder; they ascribed the effect to more active breathing patterns.

While the temperature dip of the nose has so far been ascribed to the presence of arteriovenous anastomoses (AVA) controlled by sympathetic nerves that regulate blood flow to the surface of the skin, the above scientific literature raises some questions. So far it is hard to exclude that nasal temperature observations be not based on airflow patterns or blood flow associated to muscular activity (Nakanishi & Matsumura, 2008). Nevertheless, despite the origin of the phenomenon, nose temperature changes have so far been shown to respond to stimuli of affective nature. Finally fITI is a quite expensive physiological tool for monitoring the peripheral nervous system, which is the primary reason that makes its prevalence among studies scarce.

Future directions

For the future of thermal imaging it is important to develop a consistent methodology as well as fITI user-friendly software that will be used for temperature extraction and be freely available to all users. This will overcome problems across studies related to temperature extraction, such as the size of ROI, where the ROI should be placed, whether the maximum, average, or minimum temperature should be taken into account for data analysis and in extent whether observations should be based on a number of active voxels. Some progress has been made on the above matter in terms of software development (Dowdal et al, 2006; Merla Di Donato, Romani, 2002). However literature is lacking of a methodological protocol that would be followed by the majority of fITI studies. Finally, it would be important to couple thermal observations to brain regions fulfilling a bidirectional union between the brain and the visceral organs (Kuraoka & Nakamura, 2011). Stimulation of the central nucleus (Bagshaw & Coppock, 1968; Laine, Splitler, Mosher & Gothard, 2009) as well as the corticomedial nucleus (Potegal, Hebert, DeCoster, & Meyerhoff, 1996) of the amygdala provide good cortical candidates for observing thermal changes associated with negative emotional states. Electroencephalography can also be integrated with thermal imaging since it does not occlude the participants face, thus allowing the associations of subcortical emotion-related regions with the thermal print.

2.4. Conclusions

fITI seems to provide new avenues for the study of emotions. The majority of studies have observed that in situations characterized by sympathetic activation, the temperature of the nose decreased, accounting this effect to vasoconstrictive mechanisms restricting the blood flow to the surface of the skin. Similar decreases in temperature have been observed on the upper lip or maxillary area of the face as a result of sweat gland activation. On the contrary, temperature increases have been observed on the forehead as well as in the region between the eyes and the nose. Muscular activity and increased blood flow by supra-orbital and peri-orbital vessels, respectively, are responsible for the observed temperature changes. Cooling of the cheeks has also been observed during the above mentioned phenomenon, suggested by the researchers to be caused by the adrenergic system and partially by the redirection of blood to facial regions of increased importance. Moving from human to animal studies profound decreases on tail temperature have also been observed in rats under conditioned fear. Overall the reviewed scientific evidence shows a new wireless, ecological methodology for quantifying emotional arousal and differentiating between 'rest' and affective states. Although still in its infancy, fITI provides alternative ways for addressing questions regarding ANS function in response to emotions that have so far been unexplored.

Tables & Figures

Table 1

Overview of the direction of temperature variation in the considered regions of interest across emotions.

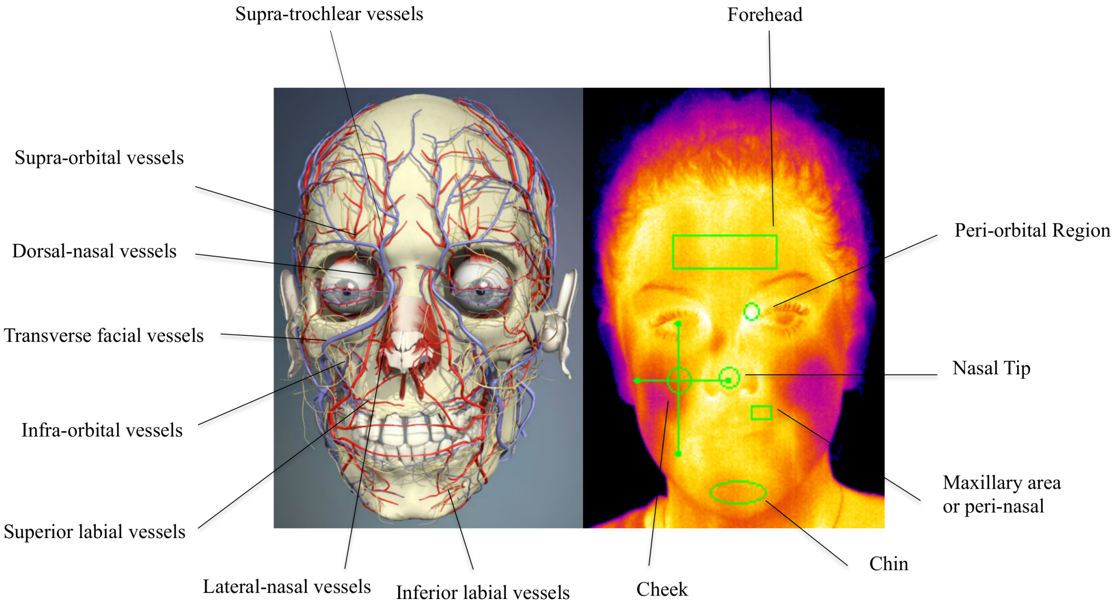
	Emotions							
	Stress	Fear	Startle	Sexual arousal	Anxiety	Joy	Pain	Guilt
Regions								
Nose	↓	↓		↑		↓		↓
Cheeks			↓					
Periorbital			↑	↑	↑			
Supraorbital			↑		↑			
Forehead	↓↑	↓		↑	↑		↓	
Maxillary	↓	↓	↓				↓	↓
Neck-Carotid			↑					
Nose	↓							
Tail		↓					↓	
Fingers/Palm		↓					↓	
Lips/Mouth				↑				

Table A.1
Overview of fITI-based studies in psychophysiology.

No.	Author	Year	N	Emotion	Experimental paradigm	Baseline	ROIs
1	Calvin & Daffy	2007	33	Stress	Driving/Mental-Loading-Task	Rest	Forehead, Nose
2	Pavlidis et al.,	2012	17	Stress	Laparoscopic drill training	Natural Landscapes	Peri-nasal
3	Puri et al.,	2005	12	Stress	Stroop Test	Rest	Supra-orbital Vessels
4	Kang et al.,	2006	9	Stress	Alphabet arithmetic task	Rest	Forehead, Nose
5	Mizukami et al.,	1990	34 (pairs)	Stress	Mother--infant separation stress /stranger exposure	Held by mother	Forehead
6	Kistler, et al.,	1998	20	Fear	Horror Movie	Pre-stimulation	Fingers
7	Merla & Romani	2007	10	Fear	Electric stimulation & Trigger	Pre-stimulation	Face, Palm
8	Kuraoka & Nakamura	2011	3 (monkeys)	Fear	Raging monkey, aggressive expressions & calls	Rest, pre-stimulation acclimatization period	Nose
9	Nakayama et al.,	2005	4 (monkeys)	Fear	Threatening Person	Rest, pre-stimulation acclimatization period	Nose
10	Vianna & Carrive	2005	12 (rats)	Fear	Foot shock chamber	Rest, pre-stimulation	All the body
11	Shastri, et al.,	2012	10	Startle	glass breaking, phone ringing	Mental task-counting circles	Peri-orbital, supra-orbital, maxillary
12	Naemura et al.,	1993	52	Startle	White Noise (45-100db)	Comparison between groups	Nasal Region
13	Pavlidis, et al.,	2001	6	Startle	Loud noise (60dB)	Rest. Sit quietly in a dark room.	Peri-orbital area, Cheeks, Neck area.
14	Gane, et al.,	2011	11	Startle	Loud noise (102dB)	Image matching task	Peri-orbital
15	Ebisch et al.,	2012	12 (dyads)	Empathy	Toy Mishap	Playing with toys	Face: Nose, Maxillary
16	Manini et al.	2013	18 (dyads)	Empathy	Toy Mishap	Playing with toys	Face: Nose, Maxillary
17	Ioannou et al.,	2013	15	Guilt	Toy Mishap	Playing with toys	Nose
18	Hahn et al.,	2012	16	Sexual Arousal	Touch on high intimate regions	Neutral face presentation	Nose, lip, peri-orbital
19	Merla & Romani,	2007	10	Embarrassment	Presence of strangers while performing a mental task	Pre-stimulation	Maxillary, Face, Palm.
20	Nakanishi & Matsumura	2008	12	Laughter	Playing	Pre-stimulation/acclimatization	Nose, Forehead, cheek
21	Pavlidis et al.	2002	12	Anxiety	Mock interrogation	Pre-stimulation	Face
22	Tsiamyrtzis et al.	2006	39	Anxiety	Mock interrogation	Pre-stimulation	Peri-orbital vessels
23	Zhu et al.	2008	38	Anxiety	Mock interrogation	Pre-stimulation	Supra-orbital vessels

Note. Table A.1 provides an overview of the studies used in the current review. Emotion labels were given according to the author’s focus of study. Moreover the table indicates the baseline used as well as the experimental approach followed to induce the emotion of interest. Finally the last column represents the region of interest in which affective observations were made.

Figure 1. Thermal representation for extraction of ROIs along with a vascular representation of the major vessels affecting the subcutaneous temperature of the face (Berkovitz, Kirsch, Moxham, Alusi, Cheesman, 2013).



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Chapter 3

3. The Autonomic Signature of Guilt in Children: A Thermal Infrared Imaging Study.

3.1 Introduction

Moral development is a process that emerges early on in human life and unfolds gradually through the interplay of the conscious self and the feedback received by social surroundings. As an important part of that development, the accompanying emotions provide children with the motivational force to attain a higher level of “fitness” between the self and the others, helping them to appreciate the link between the moral standards of their social community and their own moral behavior. Moral emotions are thought to function like a “barometer” [1], which gives the children an immediate and salient measure on the moral acceptability of their actions, whether they signal a transgression or an accomplishment of the social norms, such as shame and guilt, in the former case or pride and sympathy in the latter [2]-[4].

Given their key role in the socialization process, the vast majority of research focused more on negative than positive emotions. Since guilt and shame are thought to help children prevent aversive conducts [5]-[6], they are both considered “quintessential moral emotions”, compared to other emotions of the same family [7] (p. 447); and, between the two, guilt is judged more moral than shame [8]. Since it arises from the awareness of a wrongdoing, as opposed to the self as a bad entity [7], guilt motivates individuals to take care of other’s feelings, thus leading them to respond to the transgression by restitution and reparation rather than by avoidance and hiding. Monitoring the development of guilt from an early age could therefore be compelling in providing a reliable mosaic of child moral development at the very outset [8]-[11].

Classical theories grounded the guilt experience in the mechanism of moral internalization, focusing on early school years as critical periods for moral development [12]-[14]. Evidence [5], [15], has indicated such that children’s concern about their own wrongdoings, as well as their proneness to act appropriately for reparation, occurs even in toddler period. Even researchers reluctant to identify guilt as a distinct emotion at an early age [4], acknowledged the above evidence, as signaling the earliest steps of guilt development. According to a functionalist point of view emotions have an adaptive function and are defined not only by cognitive requisites, but also by the functions they serve in relevant contexts. Agreement now exists that discomfort showed by younger children, after causing or after thinking to have caused damage to another person can be considered as a reliable antecedent of future guilt [4]. Based on that consensus, the experience of guilt is assigned to younger children, if they show a coherent pattern of guilt-relevant behaviors in guilt-relevant situations: children must provide evidence of distress at expressive, postural and vocal level as well as of reparative behaviors at an action level, and that evidence must be provided just after a transgression happened.

To have both requisites fulfilled, past studies used a task, involving an appropriate transgression (“mishaps”) in naturalistic yet controlled conditions. Coherent results have been obtained by a number of studies in the same direction [3]-[5], [16], [17]. The mishap paradigm is considered a reliable situation for experimentally eliciting such a “blend of affective and behavioral signs of discomfort” [4], (p.462), thought to reflect future guilt.

Whereas the behavioral signs of early guilt have been identified, physiological signs have largely been ignored. However, bio-behavioral signs of higher order moral emotions should be considered. Many recent theories of emotion [18] view the autonomic nervous system (ANS) as a major component of the emotional response. The “somatic marker hypotheses” posits that somatic arousal is the connecting link between previous events and the feelings associated with the representations of those events [19]. At the neural level, the ventromedial prefrontal cortex, which is described as the focal area for guilt processing, regulates and evaluates the emotional value of sensory stimuli further projecting to the basal forebrain, and the brainstem regions, enabling the activation of physiological bodily components of an emotional response. [20]. Thus, the ANS might not only be the derivative of an emotional response, but rather a window for observing neo-cortical and sub-cortical healthy development, signifying normal moral acquisition.

Hence, it would be important to investigate ANS physiological responses in emotional research [18], [21], [22]. Only a couple of studies, to our knowledge, focused on autonomic correlates of moral emotions in early childhood. In the most recent one, [23] in a sample of children ranging from 3.7 to 4.5 years of age, positive associations were observed between cortisol levels and shame proneness, confirming a previous study evidence [24] that found a higher cortisol response being associated with a greater expression of shame. However, since saliva samples were collected in that study at six different time points to obtain cortisol responses, a quite interfering procedure was adopted not allowing contact free physiological measures.

The current study aims to contribute to research, on the physiological nature of early moral emotion by focusing on guilt. We sought to detect the autonomic signs of sympathetic arousal in children aged 39-42 months by means of online monitoring of ANS activity during the mishap paradigm [3], [17]. For this purpose and in order to preserve the naturalistic, however controlled, situation provided by that paradigm, we employed the functional infrared imaging (fIRI) technique [25]. fIRI is a contact-free and non-invasive methodology, which estimates variations on autonomic activity reflected by cutaneous temperature modulations by means of recording the thermal infrared signals released by the human body [25]-[29]. The reliability of fIRI has been proven with the use of simultaneous recordings, grounding fIRI on GSR, confirming that fIRI provides adequate detection power for physiological recordings [26]. fIRI provides a reliable tool that enables one not only to infer psycho-physiological excitement, but also differentiate between baseline and affective states [17], [31]. In particular, during emotional or physical threat a complex ‘play’ of cutaneous heat variation takes place, involving skin tissue, inner tissue, local vasculature, and metabolic activity. The above ‘acting’ mechanisms controlled by the autonomic nervous system are the driving forces of a physiological functional thermal observation. Because of its characteristics, fIRI is particularly useful for observing emotions in infancy research, when the individuals cannot express their feelings verbally and are difficult to engage in strictly controlled experimental settings [32]. In general, it represents a very advantageous opportunity for the developmental field as a whole, since children can be left free to exhibit their spontaneous behavior when adjusting to the experimental setting.

Innervations from both the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) are commonly received by all body tissue. During threat the SNS causes sweat secretions that lubricate the skin, achieving elasticity [33], [34], as well as sustaining temperature homeostasis in prolonged periods of vigorous activity [35]-[38]. Furthermore, vasoconstrictions of the blood vessels of the skin protect the body from possible hemorrhage and excessive blood loss during injury [39]-[42]. The above mentioned physiological responses, especially prevalent when emotional stimuli are present in the proximal environment [18], cause skin temperature to fluctuate as a consequence of SNS activity and subcutaneous vasoconstriction. Thus, by observing the thermal infrared signal, one can infer autonomic arousal and further differentiate between the two competing subdivision of the ANS.

The nose has been the most reliable region for detecting psycho-physiological arousal [17], [25], [31]; [32], [43]-[48]. Therefore, in the present study temperature observations for the alternating autonomic states will be based on the nasal tip. It is expected that children will show autonomic signs of distress associated with the behavioral signs of discomfort [4]. In particular, we expect a temperature drop at the nasal tip after the transgression due to subcutaneous vasoconstriction, whereas positive emotional states (playing/social interaction) are expected to have the opposite effect on nasal heat due to vasodilation and returning to autonomic equilibrium.

3.2. Methods

Ethics Statement

Full explanation of the procedure of the experiment was given to the parents of the children and all questions were answered verbally prior to the study. The parents provided their written consent and for the publication of the photograph of child 7, the guardian of the child has given written informed consent, as outlined in the PLOS consent form. Ethical approval for the study was given on January 27th 2010 (prot. 37) by the Local Ethics Committee (Comitato di Etica per la Ricerca Biomedica, Universita Degli Studi G.D' Annunzio, Azienda Sanitaria Locale – Chieti) for the study of *Emotions and the development of intersubjectivity: A functional Infrared Imaging Study.* All procedures were in line with the Declaration of Helsinki.

Participants

Participants included 20 children (8 females & 12 males; age range = 39-42 months, M = 40.38 months, SD = 0.87 months), of which 15 could be processed entirely and included in the final statistics (7 males, 8 females). Due to the ecological nature of the experiment, recordings of 3 children were interrupted by children's movements which introduced artifacts and in extent caused inconsistency among the experimental phases (e.g., they repeatedly touched their face with the hands thus affecting the temperature dynamics associated with the paradigm; or otherwise left the room searching for the parents). In addition, 2 children were excluded from the analyses because of unsuccessful engagement of the child with the toy resulting to the absence of the mishap. All children were accompanied by their mothers. They had normal cognitive development and no physical or psychological disease. Children were recruited from a database of parents who were contacted by paediatricians and covering middle socioeconomic and cultural urban areas in central Italy.

Procedure

Every child participated in a modified version of the mishap paradigm, where children are led to believe to have broken a toy previously manipulated by the experimenter [3]. The experiment took place in a room which always had a stable temperature of 23◦C, 50-60% humidity and no direct ventilation. Recordings took place across several months spanning from spring to winter. All children were dressed according to season and the majority of the thermal recordings took place in the afternoon from 4 to 5 p.m., with the exception of one participant recording taking place at 10 a.m. Before the beginning of the testing period, participants spent 10-20 min in the room for acclimatization purposes in order to allow their skin temperature to stabilize for baseline recordings [46]-[47]. It is important to note that the participants during the experimental period were seated on a chair with no body restriction. Prior to the experimental session, an adequate amount of time was provided as a 'warm up', where the experimenter played with the children using several toys, to psycho- emotionally habituate the child to the setting and the experimenter; This was performed initially in the presence of the experimenter and the parents. . After this warm up period, the child was exposed to the modified "Mishap Paradigm" [3]. During this phase the experimenter placed the 'warm up' toys back in the box and introduced the manipulated toy. The toy, a robot, was placed on a table in front of the child and the experimenter presented it as her favorite one and of particular personal value. During the experimental paradigm there were 5 distinct phases (1) "Baseline": the experimenter engaged with the child using different types of interactive games (the warm up period), followed by the illustration of the 'target toy' (a robot chosen after a pilot study) along with several activities that could be performed by the toy; (2) “Playing”: the experimenter left the child to engage alone with the “target toy” prior to stating that it was of particular personal value (experimenter’s favorite one); (3) “Mishap”: the child causes the robot to brake; (4) “Entrance Experimenter”: the experimenter re-entered the room while merely looking in silence at the broken toy for approximately 30s and then asking: “What happened? Who did that? Did you break the toy?” (5) “Soothing”: the child was told cheerfully that the accident with the toy was not his/her fault since it was already broken and that the robot could be fixed (child and experimenter fix the toy together). These phases were carried out in all experimental sessions and were videotaped for later analyses. The entire session lasted an average of 20 minutes. During the experimental process the parents could observe the experiment behind a one way mirror.

Materials and data acquisition

During familiarization and baseline recordings a variety of toys were used such as plastic animals, a puzzle, a small doll house, a magic wand and a 3D book. The target toy used during the experimental run was a black and white robot of approximately 18 cm height. It had an ON/OFF button at the back that, when switched ON, made the robot walk and play music. In addition, the hands of the toy had a button which when pressed it caused the hand-grasping mechanism to open which was the “trigger” that was causing the mishap. To be more specific, the robot had some screws loose. The appearance of the toy did not provide any visual cues to the child that it was broken since it appeared exactly the same as a new one. When the child engaged with the robot and pressed a button on the arm, the hand-like grasping mechanism was disassembling rapidly.

Data acquisition

For the thermal data acquisition a digital FLIR SC3000 thermal infrared camera was used with temperature sensitivity of 0.02K and a sampling rate of 0.02s. To cancel noise effects caused by the sensor’s shifts/oscillations the camera was blackbody calibrated and the camera was adjusted to record at 1 frame/s. For behavioral data acquisition two radio controlled cameras were used (Canon VC-C50iR) connected to two video-cassette recorders (BR-JVC). Then the two video signals were combined using a Pinnacle (liquid

6) system providing a two- or three split movies. Subsequently, with the use of the software "Interact Plus" (Mangold) behaviour of the child was encoded simultaneously with the ongoing movie. Finally, it is important to note that the results of 14 children are present in the table and not 15 since behavioral recordings did not take place for one particular child due to technical errors.

Behavioral data analysis

The children's distress was coded on a behavioral and verbal level starting from the initiation of each phase and finishing on the start of the following. The coding system for the child's responses was taken from studies of which the mishap design was practically identical to the current one [3]- [4], [10], [17]. Based on this scheme, the distress behavior of the individual is coded according to five signs: avoiding gaze (child looked away, downward); facial expression (lip rolled in), repair, verbalizations (including negative self-evaluation and confession) and bodily tension (including bodily avoidance, hunched shoulders, head lowered, arms across body, covering face, fingers in mouth). For the first four signs the presence or the absence of the behavior was considered whereas for bodily tension overall distress response was coded on a 3-point scale (0 = child shows no behavioral distress signs; 1 = child shows one sign of bodily tension; 2 = child shows two or more different signs of bodily tension). For the baseline phase, gaze avoidance, facial expression and bodily tension were only coded, for mishap, repair was also included and for the experimenters entrance phase, verbalizations were added. Moreover in the soothing phase we have interpreted as a distress sign the child's initial reaction to the fixed object (0 = child handles or touches the robot without reservation; 1 = child refuses to handle the fixed toy when prompted to do so). The rating for the children's distress was performed by two independent researchers with Kappa's alphas ranging from .72 to .80, $p < .05$. Finally, non-parametric, two related sample test was conducted in order to define if there was a significant difference in the behavioral signs of distress between baseline, mishap, the experimenter's entrance and soothing

Thermal data analysis

Thermal video preprocessing and thermal data extraction was performed using the software ThermalCAM Researcher by FLIR (http://www.flir.com) and processed according to [17]. The final data sets were analyzed using the statistical software Statistical Package for the Social Sciences, version 17 (SPSS, Chicago, IL). Prior to any data analyses, the two operators divided the thermal video into 5 experimental phases and frames were extracted every 3-5s. On average for each child at least 16 frames, equidistant in time, were used for "baseline", 8 for "Playing", 14 for "mishap", 11 for the experimenter's entrance and 15 for the "soothing" phase. Side shots of the child or frames where the face was occluded were not used since trials showed that they induced serious temperature variations in the data. Only frontal shots or frames with constant angle of view along the whole recording were used. The Region of Interest (ROI) was selected as a circular area. The radius of the circle (0.5 cm) for the temperature extraction covered the nasal tip excluding the nostrils and was the same in all experimental frames for each individual child. This was essential, since ROI radius inconsistencies might induce significant thermal artifacts.

After the extraction of data points in each condition, Mann-Whitney U tests were performed (15/individuals X 4 condition contrast) in order to observe if there was a significant difference in the temperature among the five conditions. Furthermore, for each child the mean temperature of all data points in each condition was calculated (Baseline, Playing, Mishap, Entrance Exp., and Soothing). Subsequently, using the mean values of every condition for each child, group analysis was conducted using 1x5 one-way repeated measures ANOVA. In case ANOVA yielded a significant main effect, orthogonal difference contrasts were performed comparing each condition with the mean of the previous condition and using Bonferroni correction for multiple comparisons. Because of their orthogonality, these contrasts allowed to detect independently and specifically which conditions induced a significant change on the nasal tip temperature. Raw data for each individual can be retrieved from Dryad Digital Repository.

3.3. Results

Behavioral Results

Behavioral analyses showed signs of distress accompanied by feelings of guilt particularly during the mishap phase and the experimenter's entrance (Table 1). Non-parametric, related sample tests showed that children during baseline had a significant difference in distress levels compared to mishap (bodily tension, Wilcoxon test $Z = 2.04$, $p < .05$; avoiding gaze, McNemar test, $\chi2$ (1) = 4.57 = $p < .05$; facial express $\chi2$ (1) = 4.57 $p < .05$).

Observing the experimenter's entrance phase, it seems that the level of distress did not change and children that confessed or judged themselves negatively showed more facial expressions of distress during the mishap phase ($\chi2$ (1) = 10.28 $p < .05$) as well as a more reparatory behavior when the experimenter entered the room ($\chi2$ (1) = 7.14 $p < .05$). Finally, analyzing the soothing phase, it was observed that children expressed relief touching the fixed toy without reservation; only 3 children refused to handle the toy.

Temperature Results

Individual Analyses. In order to examine at the individual level whether each experimental condition differed in temperature with respect to the previous one, Mann-Whitney U tests were performed. Each experimental condition was compared with previous temperatures, resulting to 4 comparisons for all 15 individuals (Table 2). During playing the temperature of 8 out of 15 children significantly differed from baseline, with 6 having a temperature decrease and 2 an increase. The mishap phase resulted in 12 significant temperature drops whereas during the experimenter's entrance 4 out of 15 children reached a significant temperature decrease and 7 an increase. Finally during the soothing phase 12 children showed significantly higher temperatures than the previous conditions. Based on the Mann-Whitney U tests, Figure 1 represents a summary plot of temperature direction for each child.

Furthermore, bearing in mind that measurements were made in different seasonal times and since interest is given on the change of temperature across conditions, for purposes of illustration the evolution of temperature as a function of time for each child was plotted on a scale with a range of 7 degrees and 0.5 degrees of major increment (see also Figure S1). Figure 2 shows the variations of the facial temperature in a representative child (#7).

Group Analyses. One-way repeated measures ANOVA was conducted in order to examine at the group level if there was a significant difference in the mean scores of the

15 children between the 5 conditions. Results showed that there was a significant main effect for condition [Wilks' Lambda = .404, *F* (4, 11) = 4.049, $p < .0005$, multivariate partial eta squared = .596]. Within conditions contrasts yielded a significant nasal tip temperature decrease for mishap [*F* (1, 14) = 14.178, $p < .0005$, multivariate partial eta squared = .503, (*M* = 32.63, *SD* = 1.93)], (see Table 3, 4 and Figure 3).

The mean difference between the mishap phase and baseline was 0.44 ◦C and between the mishap phase and the playing phase was 0.27 ◦C. Playing and the Experimenters Entrance (M = 33.03, *SD* = 1.79) did not differ significantly from the previous conditions (*p* >. 05). On the contrary, the soothing phase (*M* = 33.4102, *SD* = 1.83) differed significantly from previous conditions [F (1, 14) = 6.484 $p < .0005$, multivariate partial eta squared = .317]. Average temperature during the soothing phase differed 0.3 ◦C from baseline, 0.5 ◦C from playing, 0.8 ◦C from mishap, and 0.4 ◦C from experimenter's entrance. Post-hoc power analyses was performed using the G*Power software as suggested by [60] to examine whether the conducted experiment had enough statistical power to detect an effect for condition [61]. With sample size of 15 and an eta squared of .596 the study had enough power [62] to detect an effect for experimental condition (*d* = .81).

3.4. Discussion

The purpose of the present study was to characterize the autonomic response associated with guilt in early childhood. Since, from a functional perspective, guilt-relevant behaviors are exhibited by young children under the appropriate situations, the mishap paradigm was used for eliciting such kind of behaviors. Moreover, in order to preserve the children's naturalistic and spontaneous behavior, physiological assessments were made by fIRI, a non invasive method, capable of detecting the skin temperature variations expected to occur in participants involved in emotional contexts. Unlike other studies of ANS arousal in emotion, physiological recordings in the current study were made with fIRI on a continuous physiological line, supported by observations of behavioral nature. This study provides additional evidence for the reliability of the nasal region in observing affective signs and may provide ground for the physiological study of guilt in adults.

Current behavioral findings suggest that all children involved in the paradigm experienced emotional distress. According to the literature [3], [4], [10], [17], [50] the critical guilt-relevant pattern of behaviors, including expressive signals and reparative actions, were shown by all the children in the appropriate phases of the task, such as after transgression and at the entrance of the experimenter. Physiological data reflected that experience. It was observed that at the group level the temperature of the nose during mishap significantly differed from the previous conditions and particularly in contrast to baseline, dropping by an average of 0.44 ◦C. On the contrary, temperature during playing and the experimenter's entrance did not provide statistical significance in comparison to the previous conditions' temperatures. In addition, soothing also evoked a significant difference from previous conditions with the highest temperature difference observed, 0.8◦C compared with mishap. So, the paradigm confirmed to be capable of eliciting the guilt experience in early childhood. The above results are in accordance with the hypotheses.

Avoiding gaze, facial expression and bodily tension were shown by the majority of the children after the transgression occurred, and they were associated with the temperature drop of the nasal tip. We could suppose that mishap situations are experienced by the child as threatening and thus trigger the defensive physiological component of the autonomic nervous system, the 'fight or flight' response controlled by sympathetic nervous system [33], [49], [51]. This physiological phenomenon of temperature drop was shown by previous studies not to be related to heavy breathing [46] or movement artifacts [47] but it has been suggested [30], [46] to be related to the presence of arteriovenous anastomoses [50] in the nasal region, constricted by efferent sympathetic nerves. Arteriovenous anastomoses have been argued to be even more present in the skin of fingers [50] and by using fIRI it has been observed that negative emotional states induced a marked decrease in participants' fingers of up to 2 ◦C [52]. During threat or psychological stress, the SNS causes certain physiological changes to take place through the release of epinephrine in the blood stream and under this occasion subcutaneous blood vessels constrict, leading to external heat from the surface of the skin to drop [39]-[42]. Emotional or thermoregulatory sweating could not have occurred on the nasal region since no perspiration spots were detected during temperature analyses of the region of interest [47]. However it is hard to completely rule out the possibility that temperature changes were a product of altered blood flow, dependent on systemic arterial blood pressure and vascular resistance or conductance in the bed [30], since – to the best of our knowledge- no work exists on arterial pressure in children during similar experimental paradigms.

Sympathetic activity can still be inferred during the experimenter's entrance despite the fact that the temperature of the nose started rising having only a slightly lower temperature from baseline of 0.05 ◦C. This could be accounted by the fact that during this phase, where children were facing the adult, they were asked about the cause of damage and typically tried to repair the wrongdoing by apologizing or confessing. This is probably why a lower temperature than baseline was still observed, backed up also by the behavioral observations.

Lastly, it is important to note that during soothing the average temperature was 0.3 ◦C higher than baseline and 0.8 ◦C higher than mishap. According to a sensible interpretation, the child's distress was soon neutralized, or even overcompensated, during the soothing condition, which entailed social interaction and mishap restoration.

To summarize, behavioral results obtained by the study confirms the evidence of children's distress after the transgression, an observation supported by a great amount of research signaling early guilt. Physiological data showed an inverse relationship with the behavioral evidence with the temperature of the nasal tip dropping during mishap while levels of distress rose and returning to baseline temperatures after distress was reduced (see Figure 4).

This finding is consistent with a number of studies reporting significant nasal temperature drops due to unpleasant events inferring sympathetic activity (however, see [30] for seemingly opposite results in infants). For example, [45] reported a temperature drop of 0.5 ◦C as a consequence of a loud noise, [46] recorded a temperature decrease of 0.2 ± 0.1 ◦C by exposing monkeys to a threatening person, and finally, [43] reported a nasal tip temperature drop of 0.32-0.56 ◦C due to increased mental workload. Moreover, the temperature increase during soothing is also in line with the study by [44], in which they reported a temperature increase of the nose during social interaction of 0.1◦C.

All together, these results showed evidence of a coherent pattern of physiological variations underlying guilt-relevant behaviors. The findings allow to claim that young children experience distressful feeling when involved in the mishaps paradigm, providing some support to the functionalistic view of early moral development. It would be important to note that no behavioral coding was performed for the playing condition, since the scope of the study regarded the autonomic print of transgression, the adult's entrance, as well as the restoration of the wrongdoing during soothing. Although the playing phase did not reach statistical significance compared to baseline, a slight temperature decrease was observed despite the initial expectation. During play the child might have experienced a state of amusement and happiness both previously characterized by alpha- adrenergic increase [63].

In addition, observations between behavior and temperature were not based on correlation analyses but on a separate examination of the quantitative analyses of the two measures. Although physiology arises in occasions of socio-emotional or physical danger, the reason of its existence is not to cause a particular behavior but rather to support it. Behavioral engagement and the multiple strategies followed by the individual are relevant to the cognitive context of the situation [64]-[65] whereas on the contrary, only two subdivisions of the ANS apply in a variety of environmental settings [35], [66]. In line with this argument, is the experiment by [64]. Whereas individuals received epinephrine (adrenaline) shots and were exposed to euphoric and angry situations they addressed not only two different emotions but also they exhibited two different behaviors. Whereas autonomic arousal can be similar in different situations, behavior hardly ever stays constant and largely varies based on the environmental requirements. This is the reason why behavior is coded on a plethora of observations (e.g. bodily, verbal, facial) whereas physiology, only, on the competing subdivision of the ANS. Very few emotions can be specified by their physiological mark. However, if multiple physiological measures (e.g. breathing, heart rate, GSR) are taken into account [17] along with the speed of onset, intensity and duration, then it is possible that more emotions can be defined [67].

Finally, fIRI proved to be a versatile and sensitive physiological tool, extending the already existing literature of moral emotions from developmental studies to investigations of the autonomic nature of emotions. The availability of physiological measures by fIRI helps to consolidate behavioral assessments, which have been so far the only informative channel for studying moral development and self conscious emotions. fIRI is especially useful in cases where behavioral strategies are prevented, such as among paralyzed patients, when individuals can still feel emotions as prior to their injury, but can no longer show behavioral responses in the same manner [54]. In contrast to paralysis, a condition called pure autonomic failure in which the autonomic nervous system no longer regulates the heart or the internal organs, results in patients' reports of diminished emotional experiences [55]. Although these patients have no difficulty in articulating what emotion is felt [56]. ANS physiological responses seem to be more closely linked to emotional experience than to behavioral engagement; for this reason ANS measures should be essential tools in emotion research [18], [21], [22]. The simplicity of using fIRI for observing the psycho-physiological correlates of emotional experience warrants its use in developmental, comparative and evolutionary studies, particularly in the non-invasive and ecologically valid study of child behavior [57], [58]. More to the point of this study, its sensitivity to autonomic responses associated with moral experience could be particularly valuable when investigating children who have an impaired ability to exhibit some range of social-cognitive and empathic skills, such as those affected by Autism Spectrum Disorders (ASD). Moreover, fIRI might even be a valuable tool in clinical research in the early diagnosis of psychopathy since these patients have difficulty in learning morally normative responses due to impairments in emotional aversive learning [20]. Although psychopaths can show complex emotions such as embarrassment, they face great difficulties in comprehending situations imposing guilt [59]. As in many cases of pathology, though, the earlier such conditions are diagnosed, the greater is the ability to offer useful prognoses in the form of behavioral interventions and counseling.

Conclusions

Emotions have a strong physiological mark which accompanies the feeling experience. From the analyses it was observed that complex emotions such as guilt are characterized by a temperature drop, compared to baseline measures. This incidence of nasal tip thermal reduction implies sympathetic arousal through peripheral vasoconstriction related to the experience of guilt, whereas the temperature restoration during soothing could be due to parasympathetic activity and vascular restoration. Physiological states are the supporting mechanisms for the behavioral component of moral emotions. Thus, the integration of physiological elements with behavioral grounding should be crucial in the observation and interpretation of affective emotional states, as those involving the formation of the moral self.

Tables & Figures

Table 1: *Frequencies (percentage) for each variable coded and means and standard deviation for bodily tension in different phase: Baseline, Mishap, Entrance of the experimenter and Soothing (N=14).*

Variable	**Baseline**	**Mishap**	**Entrance experimenter**	**Soothing**
Avoiding gaze:	21.4	71.4	50.0	
Facial expression:	21.4	92.9	57.1	
Repair:		85.7	85.7	
Verbalizations:			42.9	
Relief to fixed object:				78.6
Bodily tension:	0.21 (0.43)	1.29 (0.83)	1.43 (0.85)	

Table 2: Mann-Whitney U tests, for all 15 individuals compared to preceding conditions (* = p < .05).

Child	**Condition**	***U***	***P***	***Z***	***r***	***Md***	***n***	***Vs.Md***	***n***
1	Play Vs.	9	.002	-3.03	-.63*	31.3	6	31.5	17
	Mishap Vs.	18	.001	-3.12	-.56*	31.1	7	31.5	23
	Entrance Vs.	31	.00	-3.50	-.56*	31.1	9	31.4	30
	Soothing Vs.	232	.08	-1.77	+.24*	31.7	17	31.3	39
2	Play Vs.	45	.71	-.391	±.09	34.9	9	34.9	11
	Mishap Vs.	34	.00	-3.42	-.60*	34.5	12	34.9	20
	Entrance Vs.	64	.03	-2.19	+.35*	35.05	8	34.8	32
	Soothing Vs.	8	.00	-5.25	+.72*	35.5	13	34.9	40
3	Play Vs.	0	.00	-3.3	-.73*	29.5	5	30.8	15
	Mishap Vs.	116	.10	-1.63	-.26	29.6	17	30.5	20
	Entrance Vs.	1	.00	-3.86	+.58*	32.95	6	30.3	37
	Soothing Vs.	3	.00	-5.67	+.74*	33.9	15	30.5	43
4	Play Vs.	33	.00	-5.16	+.73*	33.6	33	33.1	17
	Mishap Vs.	185	.04	-2.06	-.26*	33.3	12	33.6	50
	Entrance Vs.	218	.00	-2.92	-.33*	33.2	14	33.6	62
	Soothing Vs.	392	.74	-.33	±.04	33.5	11	33.5	76
5	Play Vs.	2	.00	-.38	-.73*	34.7	7	35.1	20
	Mishap Vs.	81	.00	-3.78	-.56*	34.6	18	35.1	27
	Entrance Vs.	224	.98	-.02	+.00	34.95	10	34.8	45
	Soothing Vs.	213	.06	-1.92	+.23*	35.0	12	34.8	55
6	Play Vs.	10	.27	-1.28	±.37	32.8	5	32.8	7
	Mishap Vs.	78	.74	-.34	-.07	32.7	14	32.8	12
	Entrance Vs.	51	.00	-4.08	+.63*	33.4	16	32.8	26
	Soothing Vs.	108	.00	-3.76	-.50*	32.5	15	32.9	42
7	Play Vs.	8	.02	-2.53	-.61*	34.6	5	34.7	12
	Mishap Vs.	1	.00	-5.02	-.85*	33.6	17	34.7	17
	Entrance Vs.	213	.22	-1.22	-.17	34.1	16	34.3	34
	Soothing Vs.	144	.00	-3.62	+.45*	34.6	15	34.2	50
8	Play Vs.	43	.10	-1.69	-.34	32.75	12	33.0	12
	Mishap Vs.	35	.00	-4.76	-.73*	32.3	19	32.9	24
	Entrance Vs.	282	.73	-.35	+.05	32.5	14	32.4	43
	Soothing Vs.	116	.00	-5.46	+.62*	33.1	21	32.4	57
9	Play Vs.	20	.02	-2.40	-.48*	35.8	6	36.0	19
	Mishap Vs.	6	.00	-4.73	-.78*	35.7	12	35.9	25
	Entrance Vs.	66	.00	-3.68	-.53*	35.6	12	35.8	37
	Soothing Vs.	95	.00	-3.05	+.39*	36.1	10	35.7	49
10	Play Vs.	20	.39	-.93	+.24	33.25	6	33.2	9
	Mishap Vs.	75	.13	-1.57	-.28	33.1	15	33.2	15
	Entrance Vs.	10.5	.00	-4.4	-.69*	32.75	10	33.2	30
	Soothing Vs.	161	.36	-.95	+.13	33.2	10	33.1	40
11	Play Vs.	9	.00	-2.86	-.57	33.3	5	33.55	20
	Mishap Vs.	00	.00	-5.16	-.83*	32.0	14	33.4	25
	Entrance Vs.	55	.00	-2.88	+.42*	33.8	8	33.3	39
	Soothing Vs.	145	.00	-3.67	+.46*	33.8	16	33.3	47
12	Play Vs.	4.5	.00	-2.89	+.66*	33.3	6	32.9	11
	Mishap Vs.	5.5	.00	-3.85	-.75*	32.2	9	33.1	17
	Entrance Vs.	11	.00	-5.71	-.82*	31.9	22	32.9	26
	Soothing Vs.	62	.00	-4.18	+.54*	33.7	12	32.1	48
13	Play Vs.	6	.00	-3.42	-.66*	32.3	6	32.5	21
	Mishap Vs.	11	.00	-4.80	-.76*	32.1	13	32.4	27
	Entrance Vs.	68	.26	-1.27	-.17	32.3	5	32.35	40
	Soothing Vs.	172	.47	-.716	±.1	32.3	9	32.3	45
14	Play Vs.	93	.93	-.124	-.02	33.85	6	33.9	32
	Mishap Vs.	244	.95	-.07	-.00	33.8	13	33.9	38
	Entrance Vs.	103	.00	-3.1	+.39*	33.95	10	33.9	51
	Soothing Vs.	369	.00	-5.72	+.57*	34	37	33.9	61
15	Play Vs.	70	.42	-.915	-.16	28.05	8	28.1	22
	Mishap Vs.	6.5	.00	-5.41	-.81*	27.8	15	28.1	30
	Entrance Vs.	83.5	.00	-3.46	+.46*	28.2	11	28.0	45
	Soothing Vs.	94.5	.00	-5.05	+.59*	28.4	17	28.0	56

Table 3: *Descriptive statistics for the mean temperature of each condition.*

	Mean	**Std. Deviation**	**N**
Baseline	33.08	1.95	15
Playing	32.91	2.02	15
Mishap	32.64	1.93	15
Entrance Exp.	33.03	1.78	15
Soothing	33.41	1.83	15

Table 4: *Repeated measures ANOVA for within conditions contrasts for 15 children* (* = p < .05; ** = p < .005) a computed using alpha = .05.

Source	Factor 1	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Condition	Playing vs. Baseline	.401	1	.401	2.787	.117	.166	.342
	Mishap vs. Previous	1.927	1	1.927	14.178	.002**	.503	.938
	Entrance Exp. vs. Previous	.368	1	.368	.526	.480	.036	.104
	Soothing vs. Previous	3.655	1	3.655	6.484	.023*	.317	.659
Error (condition)	Playing vs. Baseline	2.016	14	.144				
	Mishap vs. Previous	1.903	14	.136				
	Entrance Exp. vs. Previous	9.786	14	.699				
	Soothing vs. Previous	7.892	14	.564				

Figure 1: Clustered Bar Chart: Represents the temperature tendency for all children in each condition contrast for the Mann-Whitney U test.

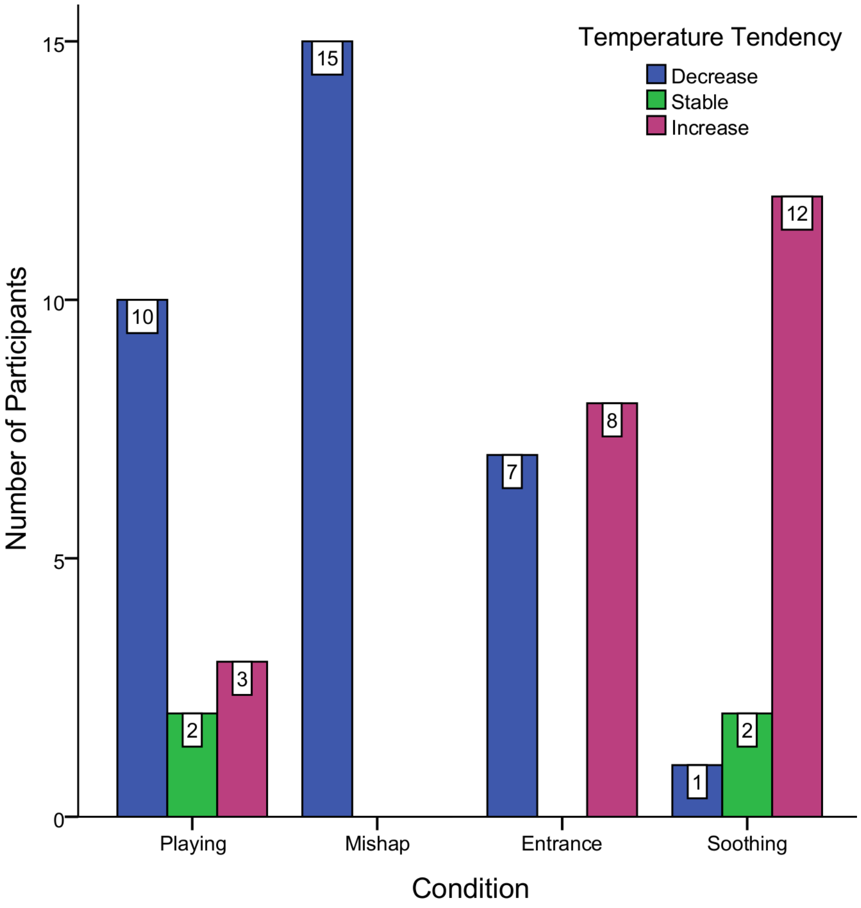


Figure 2: Five pictures of Child 7 showing the temperature change of the nasal tip: Baseline (1st frame), playing (2nd frame), mishap (3rd frame), experimenter's entrance (4th frame) and the soothing phase (5th frame). Colour change from lighter to darker shades signify the temperature drop.

Figure 3: Group graph: Z Mean temperature for each condition.

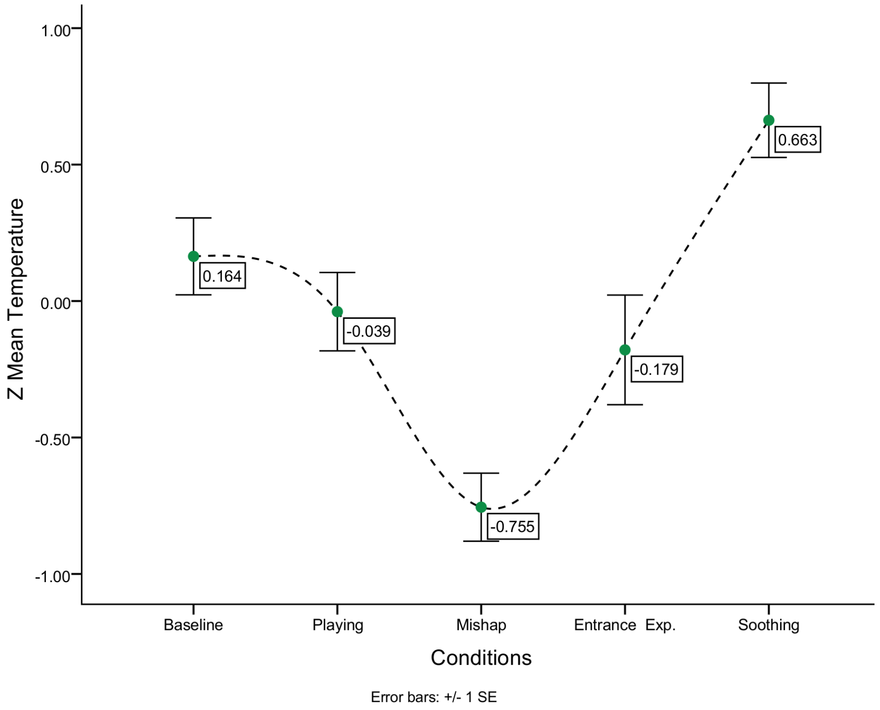


Figure 4: Group graph: Z mean behavioral distress signs and Temperature change for Baseline, Mishap and Experimenter's entrance.

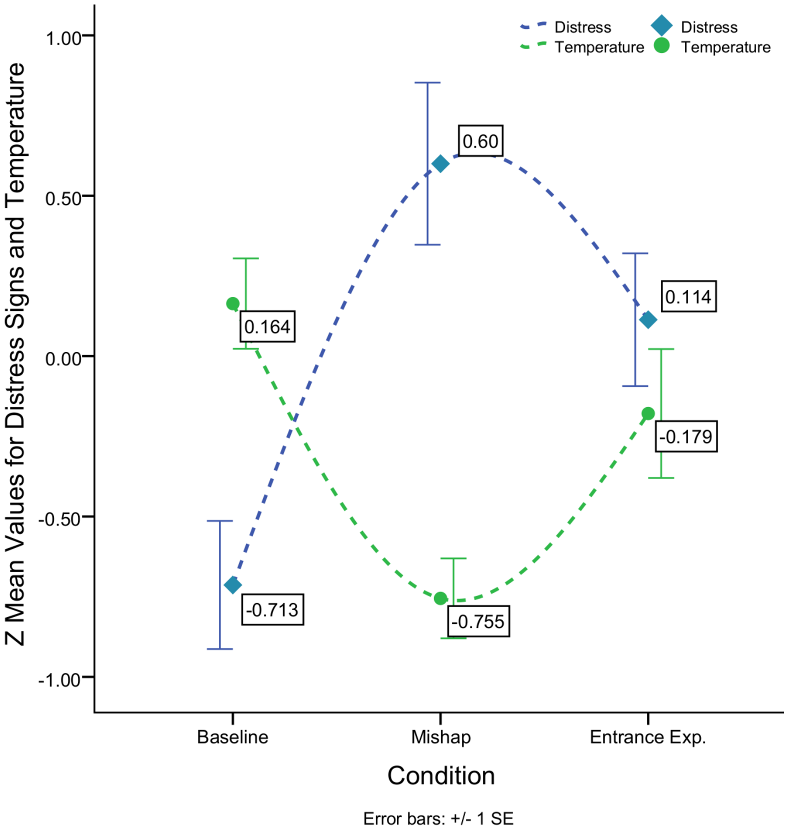
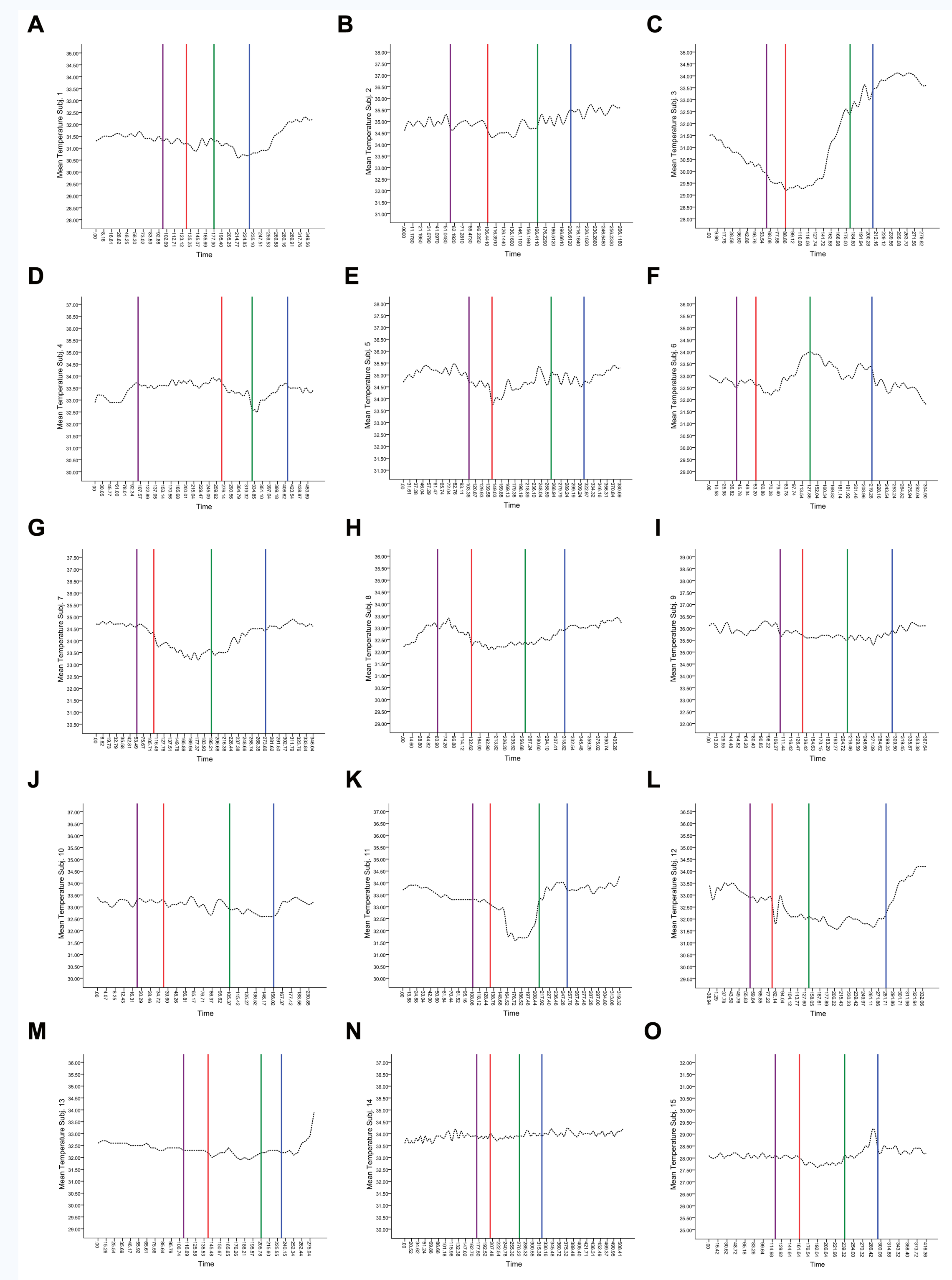


Figure S1. Line Graphs. Graphs representing the evolution of temperature over time for each child. The coloured vertical lines represent the onset of each condition (purple = playing, red = mishap, green = entrance experimenter, blue = soothing).



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Chapter 4

4. Facial Imprints of Autonomic Contagion in Mother and Child: A Thermal Imaging Study

4.1. Introduction

Seeing one's own offspring in distressing situations is a rather ordinary occurrence in everyday life. A sense of empathy with the child's feelings helps parents to understand the child's needs and to provide congruent responses. In fact, a mother's ability to empathically share offspring's emotional feelings in distressing situations is considered integral to the creation of primary affective bonds and a healthy socio-emotional development (4, 17, 32, 37, 41, 42, 48). What neurobiological mechanism subtends maternal empathy in humans? In particular, does sharing of autonomic arousal accompany a mother's affective sharing of her offspring's distress? Autonomically-mediated visceral responses are proposed to be strictly related to the experience of emotional feelings (10, 11, 12, 22, 23, 29, 31, 47).

The sympathetic and parasympathetic divisions of the autonomic nervous system represent the principal channels of interaction between the brain and bodily organs, and have complementary roles in the achievement of homeostasis and the regulation of physiological responses to emotional stimuli (5, 10, 24). It is therefore plausible that the vicarious response of empathy, generally referred to as a common neural coding of the perception of one's own and the other individual's feelings underpinning a sharing of affective experiences (1, 13, 18, 19, 40, 44), also embodies a direct sharing of changes in body physiology between the involved individuals (10, 11, 12, 13, 40). The present study is an extension of a previous one aimed at investigating whether a mother's empathic sharing of her offspring's distress is accompanied by physiological sharing of autonomic responses (16). For this purpose, facial thermal imprints of mother and child dyads were simultaneously recorded in an ecological context while mothers observed their children when involved in a distressing situation. We used thermal infrared (IR) imaging which estimates variations in autonomic activity reflected by cutaneous temperature modulations by means of recording the thermal infrared signals spontaneously released by the human body (20, 34, 38, 39, 43). Facial temperature is mediated and regulated by sympathetic and parasympathetic activity, which works to preserve the body homeostasis in the human physical and psychological functioning (2), and therefore is especially active when emotional stimuli are present in the proximal environment (29).

4.2. Method

Participants

Thirteen mothers (age 31-46 years) and their typically developing biological children (5 male, age 38 - 42 months) participated in the experiment. Two out of 13 mother child dyads were excluded from further data analysis, since the toy was not broken during the experiment (see Procedure section). Inclusion criterion for both mothers and children was the absence of any overt physical, psychiatric or psychological disease. All participants observed standard preparation rules for thermal imaging. The study was approved by the local Ethics Committee. Written informed consent was obtained from all participants after full explanation of the procedure of the study.

Procedure

Prior to testing, each subject was left to acclimatize for 10 to 20 minutes to the experimental room and to allow the baseline skin temperature to stabilize. The recording rooms were set at standardized temperature (23 °C), humidity (50 - 60%), and without direct ventilation. Participants comfortably sat on a chair during both acclimatization and measurement periods, without any restriction to body movement. Before the start of the experiment the children underwent an adequate familiarization period for psychological habituation to the setting and the experimenter, first in presence of their mothers, followed by neutral interaction with the experimenter alone. After a neutral baseline period of interactive activities with the experimenter, children were presented with a potential stressful experience elicited by the "mishap paradigm" (7). More specifically, children were invited to play with a toy, which was previously manipulated to break in the child's hands when playing with it, thus suggesting that the child accidentally broke the toy. The toy was introduced by the experimenter as her own favorite. Distinct phases could be distinguished in the paradigm: 1) "presentation" (the experimenter demonstrated the toy); 2) "playing" (the child played with the toy, while the experimenter left the room for 1 minute); 3) "mishap" (child broke" the toy); 4) "re-entrance" of the experimenter (the experimenter did not say anything for 30 seconds and merely looked at the broken toy); 5) "soothing" of the child (the experimenter cheerfully indicated that the toy could be fixed and that the breaking was not the child's fault). Mothers were invited to observe their children in interaction with the experimenter through a one-way mirror from a separated room, while naïve about the specific content of the experiment.

Materials and data acquisition

Thermal IR imaging was performed by means of two digital thermal cameras (FLIR SC3000, FlirSystems, Sweden), with a Focal Plane Array of 320 x 240 QWIP detectors, capable of collecting the thermal radiation in the 8-9 μm band, with a 0.02 second time resolution, and 0.02 K temperature sensitivity. The thermal camera response was blackbody-calibrated to null noise-effects related to the sensor drift/shift dynamics and optical artifacts. Thermal images of the faces of the mother and the child were simultaneously acquired along the whole experimental paradigm. Sampling rate for thermal imaging was set at 10 frames/sec. Behavioral recordings of the children took place through two remote-controlled cameras (Canon Vc-C50iR). Video-signals were sent to two videorecorders (BR-JVC) and the resulting movies were subsequently mixed by a Pinnacle system (Liquid 6) to have a two- or three-split image. Subsequently, the movies were processed in a video reading lab by specialized software (Interact Plus, Mangold) that allows coding behavior in synchrony with the ongoing movies of the children during the experiment. The toy presented to the children in the "mishap paradigm" was a black and white colored robot with a height of approximately 20 cm. When turning on the robot with a switch on its back it started to walk and play music. Both hands of the robot could be opened and closed by means of pressing/relieving a button. The hands of the robot were prepared to break when manipulated by the child. The robot could be repaired only by the experimenter. During neutral interaction between the experimenter and the child, other toys were presented, such as a puzzle, a magic wand and a pop-up book.

Behavioral data analysis

Behavioral and verbal signs of children's distress during the experiment were observed according to a reliable coding system used in previous research and in the same context (7, 27, 28). According to this scheme, the child's behavior is coded according to 5 main categories (gaze direction, facial expression, bodily tension, actions and verbalizations) and various parameters (frequency, duration, latency) by two independent raters. Following from the notion that different combinations of signs may be indicative of distress, in previous studies of guilt and shame reactions to mishap paradigm and task failure (28, 33) distress has been scored categorically by restricting the number of criterion signs to five (eye/face, head/body, arms/hands, action and verbal). Thus, in the mishap and entrance phase, distress in response to mishap was defined "absent" if the child was not affected by the mishap in any way, "low" if the child showed behaviors included in one of the five coded signs, "medium" if the child showed behaviors included in two or three of the five coded signs, and "high" if the child showed behaviors included in at least four of the five coded signs.

Thermal data analysis

A visual inspection of the changes in facial thermal imprints in 10 mother-child dyads was performed to qualitatively investigate the autonomic responses of mother and child throughout the experiment. This visual analysis was followed by a quantitative estimation of temperature variations in relevant facial regions of interest in 11 mother-child dyads. Thermal facial imprints and variations in cutaneous temperature of facial regions of interest in children and their mothers were analyzed using custom made Matlab programs (http://www.mathworks.com). To chase a cluster of pixels corresponding to the same region on the face, we corrected, whenever possible, for translation of the face in the thermograms, which arose from body movements before analyzing changes in facial skin temperature. In case of marked rotation of the head, we skipped to the next frame in which participants restored their initial position. We corrected for the displacement between images frame by frame, using anatomical landmarks based on the participant's nose profile (15). In order to quantify thermal variations and their correlation between children and their mothers, changes in cutaneous temperature for the nasal tip were investigated. This region was selected according to previous studies in humans as well as primates (30, 36, 43) as associated with the activation of the sympathetic nervous system by emotional or distressing stimuli (30, 34, 35, 36, 43). More precisely, thermal changes of the nasal tip may reflect sympathetic alpha adrenergic vasomotor effects. Furthermore, sympathetic stimulation of the blood vessels can also have smaller vasodilatory effects via cholinergic and beta-adrenergic receptor action (45). First, we assessed at the intra-individual level whether the facial skin temperature did not vary significantly or presented drifts during a 90 second baseline period immediately preceding the experiment, thus providing evidence for proper participants acclimatization. Second, in order to investigate the presence and timing of the change in skin temperatures following stimulus presentation (i.e. the onset of the experimental phases), we carried out multiple comparison tests between the 10-second pre-stimulus period (from 10 to 0 s before stimulus presentation) and each of the six 10-second post-stimulus periods. Analysis of variance (ANOVA) results rejected the hypothesis of equality of the means of the distributions. Dunnett's t-test showed that stimulus-related skin temperature variations occurred within the first 10 seconds and lasted from 20 to 30 seconds for the mishap, entrance and soothing phase. Therefore, for further analysis of the individual mother-child dyads as well as of the group data, we decided to take into account 5 representative frames for each experimental phase in which emotional modulation took place (mishap, entrance, soothing), as closest in time as possible to 6, 12, 18, 24, 30 seconds after the beginning of each phase. This procedure also allowed to deal efficiently with the participants' motion and vocalizations in the ecological experimental setting, by excluding frames affected by short-lasting motion or vocalization artifacts. Thus, a total of 15 frames (data points) were obtained for each participant for the analysis of the experimental phases. Similarly, 15 frames (each frame taken every 5 seconds, and not affected by the abovementioned short-lasting artifacts) from a neutral baseline period of 90 seconds immediately preceding the experiment were obtained. The nasal tip temperatures of the children were manually extracted from the available thermal images. For extraction of the mothers' nasal tip temperature, a tracking algorithm was applied to the thermal videos to ensure the proper localization of the defined facial ROI (nasal vestibule area) on each of the processed frames of the experimental phases (baseline, mishap, entrance, soothing). The tracking algorithm is based on the 2-D cross-correlation between a template region, chosen by the user on the initial frame, and a similar ROI in a wider searching region, expected to contain the desired template in each of the following frames. ROI average temperature distributions were computed in order to extract the time courses. For analysis, ROI average temperatures of the mothers were extracted at the time points corresponding to those of the selected thermal images of the children. In order to verify whether there was a significant modulation of skin temperature in children and their mothers during the experimental phases and the baseline period in these facial region of interest, group ANOVAs were performed with the thermal values at the 15 selected time points according to the procedure described above in the facial region of interest as within-subject variable. Group correlation analyses (Pearson coefficient) analysis was performed on the thermal time courses of the selected regions of interest for the experimental phases in which the emotional modulation took place (mishap, entrance, soothing), investigating quantitatively whether the individual mother-child dyads showed a synchronicity in autonomic activity. In order to verify whether correlations between autonomic parameters were specific for the experimental phases, that is, situation specific, the same procedure was applied to a baseline period of neutral interaction between the experimenter and child immediately preceding the experiment. In order to standardize the individual time courses, the thermal value of each selected data point in the time course was converted to a z-score. Subsequently, the standardized individual thermal time courses were averaged separately for the children and the mothers. Correlation analysis was performed between the averaged time courses of the children and the mothers.

In order to account for the possibility that the observed thermal variations in an empathic situation could reflect respiratory alterations, the mothers' temperature dynamics of the nasal tip were correlated with respiratory alterations. For the extraction of the breathing signal, a tracking algorithm was applied to the thermal videos to ensure the proper localization of the defined facial ROI (nasal vestibule area) on each of the processed frames of the experimental phases (mishap, entrance, soothing). The tracking algorithm is based on the 2-D cross-correlation between a template region, chosen by the user on the initial frame, and a similar ROI in a wider searching region, expected to contain the desired template in each of the following frames. ROI average temperature distributions were computed in order to extract the time courses of the nasal breathing signal of the participating mothers. Once the breathing signal was extracted and opportunely band-pass filtered (0.25-0.6 Hz), the duration of breathing cycles (in seconds) was evaluated using an algorithm based on zero-crossing detection of the de-trended breathing signals. The obtained breathing cycle series were smoothed using a moving average (span of 8 signal samples). Data extraction and following analysis were developed by homemade Matlab algorithms (the Matworks Inc.).

Prior to the computation of Pearson correlation coefficients, we followed the same procedure as described above for calculating the correlation between facial temperature dynamics of mothers and their children. Thus, for every mother we took into account the respiratory cycle duration at 5 equally distributed time points for each of the experimental phases (mishap, entrance, soothing). Then, correlations between the resulting 15 data points representing the duration of breathing cycles and the 15 data points representing the nasal tip temperature were computed for the individual mothers. A group analysis was also performed. In order to standardize the individual respiratory series, the value of each of the 15 data points was converted to a z-score. Subsequently, the standardized individual respiratory time series were averaged for the group of mothers. Correlations were calculated between the average nasal tip temperatures and average respiratory cycle durations. Furthermore, multiple regression analysis was performed with both nasal tip thermal signal of the children and mothers' respiratory activity as independent variables, and nasal tip thermal signal of the mothers as dependent variable. In order to verify whether there were significant respiratory alterations in the mothers during the experimental phases, a group ANOVA was performed with the 15 respiratory cycle duration values as within-subject variable.

4.3. Results

Behavioral results

As expected, behavioral data confirmed a significant increase of children's distress during the experimental phases, that is, after the mishap. According to the categorical scores, distress levels across the children varied between medium and high.

Visual analysis of facial thermal imprints

A visual inspection of the changes in facial thermal imprints was performed to investigate the presence of appreciable signs of autonomic responses of mother and child throughout the experiment. As to the child, no appreciable modulations were detected regarding facial skin temperature distribution during the presentation and playing phase. However, after the mishap (i.e. after the breaking of the toy), a sympathetic reaction could be observed, reflected by a sudden and wide-spread decrease of face temperature, especially in the maxillary area and nasal tip as previously found in human as well as macaques (30, 34, 35, 36, 43). This sympathetic reaction was accompanied by sudomotor response (34), which is in the maxillary area likely regulated by sympathetic postganglionic cholinergic activity, whereas the decreased skin temperature in the nasal tip could reflect peripheral vasoconstriction due to alpha-adrenergic activity. These sympathetic responses were maintained after the entrance of the experimenter. During the soothing phase, the sudomotor response in the maxillary area was initially maintained, whereas the temperature of the nasal tip soon increased, likely reflecting a withdrawal of the sympathetic alpha-adrenergic vasoconstrictor effect. This initial response was followed by a more generalized face temperature increase and the extinction of the sudomotor response, up to re-establishing the baseline state. Moreover, an over-response of nasal tip temperature was observed, compared to the start of the experiment. Concerning the mother, no appreciable modulation of skin temperature distribution was detected during the presentation and playing phase. After the mishap as well as after the entrance of the experimenter, the same thermal variations observed in the child were detected in the mother, although more intensely in both cases. During the soothing phase, the mother showed a gradual and generalized increase of facial temperature with extinction of the sudomotor response in the maxillary area, re-establishing the baseline state. Moreover, like children, mothers showed an over response of nasal tip temperature, compared to the start of the experiment.

Mother and child in synchrony

ANOVAs with the temperature values of the facial region of interest (nasal tip) at the different time points as within-subject variable showed a significant modulation of temperature during the emotionally charged experimental phases (mishap, entrance, soothing) for the child [$F(14,140) = 5.605$, $p < 0.001$] as well as for the mother [$F(14,140) = 2.339$, $p < 0.01$]. No significant modulation of temperature was detected during the baseline period, neither for the child [nasal tip $p > 0.9$], nor for the mother [nasal tip $p > 0.4$]. Group analyses for the phases in which the emotional modulation took place (mishap, entrance, soothing) showed significant positive correlation coefficients between thermal fluctuations of mother and child for the nasal tip ($r = 0.87$, $p < 0.0001$).

With respect to the neutral baseline condition, no significant correlation could be found at the group level for the nasal tip ($r = 0.13$, $p > 0.6$), suggesting that the observed parallelism in thermal variations between mother and child also at the group level was specific for situations with an emotional valence. Example of mother-child dyad temperature variation is shown in fig. 1. Group results are graphically presented in fig. 2.

Control analysis for respiratory effects on thermal variations

Correlation analyses investigating the relationship between thermal variations on the nasal tip and nasal respiratory variations in the individual mothers did not yield significant correlations ($p > 0.05$). Group analysis confirmed the lack of a correlation between nasal temperature dynamics and respiratory activity in the mothers ($p > 0.7$). Furthermore, a multiple regression analysis showed that nasal tip temperature variations in the mothers were statistically independent from the mothers' respiratory activity (Beta = 0.02, t = -0.17, $p > 0.8$), whereas the relationship between nasal tip temperature variations in the mothers and those in the children remained significant (Beta = 0.91, t = 7.963, $p < 0.001$). ANOVAs with the respiratory cycle duration values as a within-subject variable failed to show a significant modulation of respiratory activity in the mothers during the experimental phases ($p > 0.1$).

4.4. Discussion

The present study provides two main results. First, facial thermal imprints of the mothers suggest that observation of their child's experience of distress induced significant emotional arousal mediated by the autonomic nervous system. The facial thermal modulations observed in the mothers were surprisingly similar to those observed in the child. Second, facial thermal modulations of the mothers correlated with corresponding modulations of their children at the individual as well as at the group level. Control analyses showed that the thermal variations observed in the mothers in an empathic situation are unlikely to reflect respiratory alterations or other short lasting artifacts throughout the experiment. Although both vasomotor and respiratory activity could be modulated by emotional stimuli, facial thermal variations were statistically independent from mothers' respiration and no significant alterations of respiratory activity were detected in the mothers during the experimental phases. Furthermore, segments in the thermal time courses corrupted by motion or vocalization artifacts were excluded from quantitative data analysis and would not be able to explain the observed parallelism between mother and child.

Thus, mother-child dyads showed a significant and situation-specific synchronicity between the autonomic reactions individually exhibited by each partner. These results, showing a synchronism between mothers' and children's autonomic responses, offer direct evidence for the affective aspect of empathy as an embodied vicarious process. These findings are also consistent with the notion that both the psychological and the neural components of emotional feelings are essentially integrated with autonomic-visceral changes (8, 9, 10, 11, 12, 22, 23, 29, 31, 47).

The present study provides reliable measures of autonomic responses recorded simultaneously for both distressed children and their empathizing mothers, without the disadvantages of most of the physiological methods when applied to psychological domain, including the poor practicability and psychologically demanding character of the measurement equipment. By means of thermal IR imaging, physiological correlates of emotional reactions were investigated in an interactive and ecological experimental context without interfering with spontaneous behavior and without age restrictions (30, 36, 43). This ecological context has the advantage of obtaining more valid and generalizable data than those collected in artificial laboratory settings. This also suggests important applications of the thermal IR imaging technique for providing data that add to developmental, comparative and evolutionary research on emotion in humans as well as non-human individuals (3, 13). Some other issues should be noted. Although the present results show plausible evidence for emotional sharing between mother and child, testing whether similar patterns of physiological responses emerged in the mother if she had broken the toy herself, could properly support this evidence. Likewise, testing the mothers against other categories of people could support the maternal nature of this emotional sharing. Differences both in intensity and in synchrony of autonomic responses could be expected between mothers and other groups. Differences should also be expected between women who are the mothers of the observed children and women who are not, the latter supposed to be less emotionally tied to the child or to be less familiar with the child's typical behavioral signs of distress. In sum, based on the present results, further studies using relevant control groups are encouraged in order to test specific hypotheses. Furthermore, mimicry of facial muscular responses have been found to be predictive for self-reported empathic experiences and are related with variations in facial temperature as well (21, 25, 46). It would be a relevant issue for future studies to integrate these different types of measurements in order to gain more insight on the interrelationship among different levels of empathic responses, like the motor, autonomic and experiential ones(26).

In conclusion, the present results pave the way for a more comprehensive approach to the investigation of the neurobiological basis of emotional parent-child relationships as a multidimensional phenomenon. Supporting the hypothesis that empathy also embodies a direct sharing of visceral autonomic responses, we found a close and specific parallelism between the autonomic variations of mothers observing their children in a distressing situation and those occurring in children themselves. Since this sharing is assumed to represent the most basic and direct level of empathy (14), the present results provide reasonable evidence for a crucial and still poorly explored aspect of the phenomenon under scrutiny.

Finally, because of the contact-free nature of thermal infrared (IR) imaging, its successful application to psychological research suggests that it could be particularly useful when investigating the neurobiological basis of behavior, especially in populations difficult to involve in controlled and artificial experimental settings, like children. It also allows to study people in ecological settings without interfering with spontaneous behavior, providing more valid and generalizable results.

Tables & Figures

Figure 1: Facial Imprints of one of the mother-child dyads

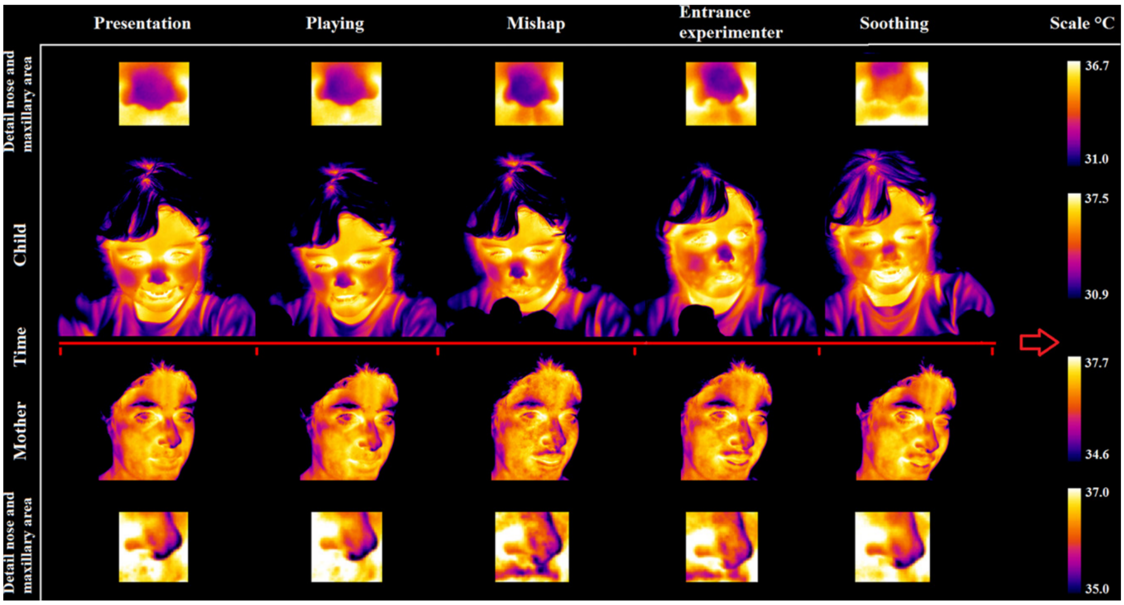
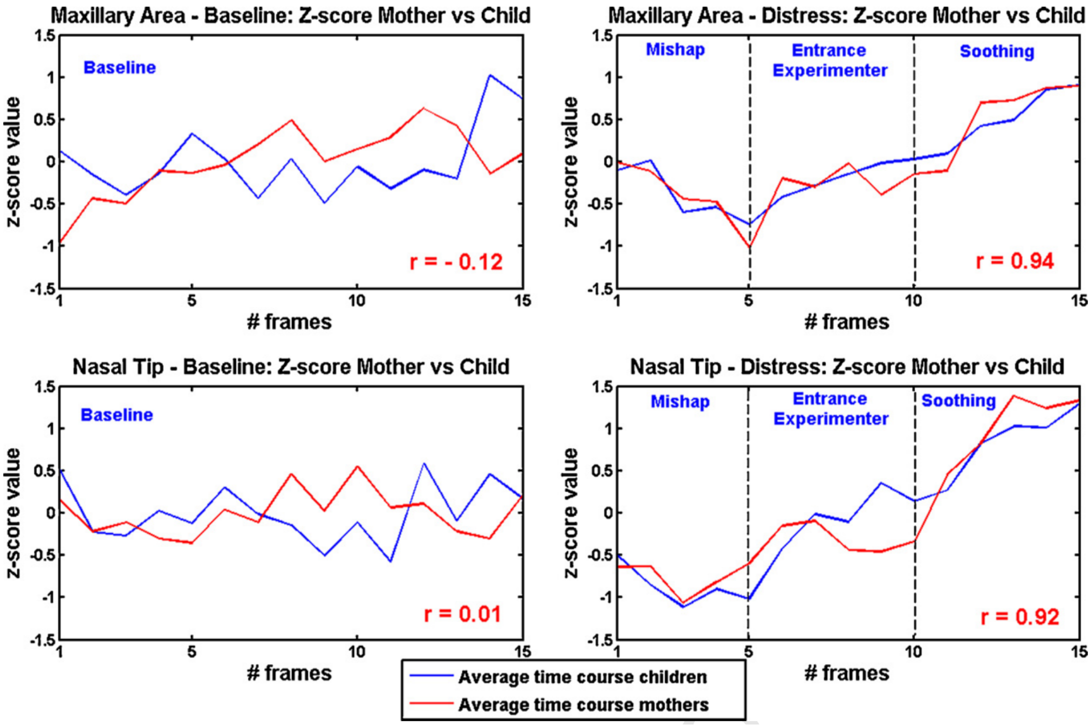


Figure 2: Graphical representation of group temperature variations of the nasal tip and maxillary area during the experimental phases as well as during the neutral baseline period.



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Chapter 5

5. Proximity and Gaze Influences Facial Temperature: A Thermal Infrared Imaging Study.

5.1 Introduction

The way people communicate and engage in emotional and intentional exchanges, takes place through the recognition of the subtle cues conspecifics generate (Freeth, et al., 2013). Non-verbal social cues communicate information that influences and challenges the way in which the receiver interacts with the environment (Patterson 1995). Gaze (Frischen, et al., 2007) and interpersonal distance (Bailenson, et al., 2001) are primary sources of social understanding, conveying a range of information regarding intentions (Nummenmaa, Calder, 2009), interpersonal relationships (Evans, Howard, 1973; Little, 1965), character (Argyle, et al., 1974; Sodikoff, et al., 1974), culture (Hall, 1966; Watson, 1970) as well as mental health and emotional state (Aziraj, Ceranic, 2013; Oliver, et al., 2001; Freeth et al., 2013). Competence in interpersonal interaction is important for reproduction and survival and therefore important from an evolutionary perspective. At the cognitive level this is achieved through the recognition of opportunities and threats, whereas at the behavioral level, by the selection of social strategies for exploitation or avoidance (Bodenhauzen, Hugenberg, 2009). The autonomic nervous system (ANS) is an integral part of the social engagement process and alters its activity to foster behavioral strategies of threat engagement or non-emergency vegetative states (Porges, 2001).

Gaze, characterized as affording a “language of the eyes” (Frischen et al, 2007, p. 694), can communicate to the receiver a range of mental states, such as intentions, emotions, desires and beliefs. Across species direct or averted gaze is a crucial communicative element, where the perception of being perceived constitutes an important tool for survival. Emery (2000) states that gaze perception has evolved as a form of warning, informing the organism that a predator is attending to it. Many animals respond to being stared at with exhibitions of fear and submissive behavior, showing that the identification of direct gaze is perceived as a warning mechanism (Schwab & Huber, 2006). Neuroimaging data have shown that the amygdala, a major structure for emotional processing, responds when individuals observe images of others engaging in direct gaze, rather than when they look somewhere else (Kawashima et al., 1999). Furthermore, the peripheral nervous system seems to be affected by gaze direction. The participants' skin conductance increases when observed by another, an indication that long periods of eye contact are perceived as threatening or aggressive (Nichols, Champness, 1971; Hoffman et al., 2007; Hietanen et al., 2008). People seem to be highly sensitive to being looked by others, showing finely tuned elements for the detection of others' gaze (Baron-Cohen, 1995). Visual search experiments have shown that less time is taken to find eyes that are directed towards the observer than eyes that are looking at another target (Conty, et al., 2006). Direct gaze captures the attention of the observer and according to fMRI data what has been specified as the "eye direction detector" by Frischen et al., (2007) is located in the fusiform gyrus of the brain (George et al., 2001; Wojciulik, et al., 1998). Moreover, during transitory gaze states, activity has been observed in the superior temporal sulcus activated when an approaching person shifts attention to the participant rather than when mutual gaze is disengaged (Pelphrey, et al., 2004). Moving from the normal population to clinical disorders, people with autism are reported to find eye contact aversive (Dalton et al., 2005). In fact, when compared to controls during tasks in which they are asked to explore the eye region of the face, people with autism show increased skin conductance as well as greater activity in the amygdala and fusiform gyrus. Their avoidance or dislike of eye contact suggests a strategy of physiological regulation (Dalton et al., 2005). Similar preferences and srtategies are observed in people with social phobias (Horley, Williams, Gonsalvez, & Gordon, 2002).

The study of proxemics dates back more than four decades (Hall, 1959; Sommer, 1959). Hall (1966) defined interpersonal space in four different categories Intimate (0-46 cm), Personal (45-120 cm), Social 1.2-3.5 m) and Public (3.5 m+). Interpersonal space seems to be affected by a variety of individual and cultural differences (Hall, 1966). Women have smaller personal space when interacting with other women (Larsen, LeRoux, 1984) and, in contrast to males, dislike lateral intrusions into their personal space (Fisher, Byrne, 1975). Sanders (1978) showed that women maintain a larger personal space during menstruation. Moreover, in the United States, Malaysia, Spain and Chile, irrespective of their country of origin and gender, people preferred to be touched by a female rather than by a male (Willis, Rawdon, 1994). To some extent Spanish men were the most tolerant in terms of being touched by other males, whereas Malaysians irrespective of gender were the least tolerant of being touched. Women from the United States had the highest tolerance in terms of being touched by the same gender. In the same line of research, Little (1968) asked Americans, Swedes, Greeks, Italians and Scots to place a doll at the distance in which they would normally interact with another individual. Scots placed the doll at the greatest distance with Greeks placing the doll nearest. Age as well as prior knowledge about the forthcoming experience seems to also play a defining role in interpersonal distance. Older individuals prefer to sit further away from the interviewer regardless of expectations about the pleasantness or unpleasantness of the situation (Feroletto, Gounard, 1975). On the other hand, younger individuals are affected mainly by their expectations about the situation. Perceived violence and level of criminality also affect personal space. People are generally less reluctant to sit next to an individual who has never committed a crime than to violent and non-violent offenders (Skorjanc, 1991).

Four main theories have been proposed to explain why people protect personal space (Bell et al., 1996). "Arousal theory" argues that positive or negative emotional arousal is the critical factor determining the boundaries of personal space. "Behavioral constraint theory" argues that personal space is needed in order for the individual to be able to express and act at will with no space constraints. "Overload theory" argues that personal space is needed to avoid sensory overload. "Stress theory", which is the last theory proposed by Bell et al. (1996) links the three theories outlined above, arguing that personal space is needed so that the individual does not get stressed about close proximity. Studies with clinical populations have shown that schizophrenic patients, compared to controls, require larger personal space (Deus, et al., 2006) and people with anxiety, compared to individuals with psychotic disorders, leave more space between themselves and the experimenter (Aziraj, Ceranic, 2013).

Personal space seems to expand or shrink according to the intimacy level entertained by participants. According to equilibrium theory, mutual gaze and personal space are two inversely related social behaviors (Argyle and Dean, 1965) modulated by intimacy: interpersonal space increases in order to balance out the effects of direct gaze. When interacting with avatars in a virtual environment, people leave more space between themselves and the virtual agent when direct gaze is involved; when the avatar invades their personal space, participants move further away (Bailenson, et al., 2003). Data collected with electroencephalography and other psychophysiological measures is consistent with the above behavioral findings. When a male experimenter was looking at a male participant from a close distance, arousal was at its peak compared to when gaze was averted. In addition, when distance was increased arousal diminished; nevertheless, direct compared to averted gaze always caused greater arousal independent of distance (Gale, et al., 1975). McBride et al., (1965) found that galvanic skin response (GSR) increased as a function of proximity and frontal confrontation. Similar results were observed by Nichols and Champness (1971). Finally, in a study that examined these effects in a clinical population, highly anxious women avoided gaze contact and exhibited backward head movements in response to male avatars who showed direct gaze. These behaviors were exhibited independently of distance (Wieser, et al., 2010).

Porges' Polyvagal theory is one of the most influential interpretations on the role of the ANS in social engagement (Porges, 2001). Through evolution the ANS retained three neural pathways whose hierarchy reflects their phylogenetic origins. On the top of this pyramid is a) the social engagement system that is part of the parasympathetic nervous system (PNS) (Cannon 1928), which inhibits more primitive structures of b) mobilization (e.g. fight-or-flight) and c) immobilization (e.g. feigning death, behavioral shutdown "syncope"). Having its main control component in the cortex, the social engagement system controls brainstem nuclei responsible for communicative movements such as eyelid opening, facial muscles contraction, head turning, pharyngeal and laryngeal muscles activation, middle ear muscles activation, as well as activation of the mastication muscles. These facial muscles have been reported to be dysfunctional in several types of psychopathology such as depression, autism, antisocial personality disorders and post-traumatic stress disorders. Beside head muscles, which are the "beacon" of human interaction, other structures such as the cardiopulmonary and the sympathetic-adrenal system alter their activity to support social demands and physiological relaxation. In situations of threat the 10th cranial nerve (vagus) that controls the heart is disengaged, providing an immediate increased cardiac output for mobilization of the organism without the need for activating the costly sympathetic adrenal-system. It is only after prolonged challenges that the sympathetic nervous system takes action. Mammalian evolution allowed the rise of this efficient mechanism that enables not only fast mobilization, but also fast physiological restoration, as re-engagement of the vagal nerve inhibits sympathetic inputs to the heart (Valhoutte & Levy, 1979). People with social or affective disorders do not show the same efficiency in dealing with environmental stressors as emotional arousal seems to engage lower, more primitive structures associated with immobilization and energy saving, rather than primary physiological responses such as heart mobilization and sympathetic engagement. Low cortisol reactivity has been linked to post-traumatic stress disorders (Yehuda et al., 1996), schizophrenia (Jansen et al., 2000), as well as to child neglect and abuse (De Bellis et al., 1994).

The majority of studies that have examined social attention and proximity were conducted decades ago with only a few managing to exercise full experimental control over the variables of interest. In fact to date social attention research has largely been conducted in non-realistic experimental settings (Freeth, et al., 2013) without the agent being physically present (Risko, et al., 2012). Furthermore, only few studies have addressed physiological elements of social arousal despite the fact that somatic arousal defines behavioral engagement strategies (Damasio, 1996). The present study aims to measure physiological responses in a more ecological experimental setting using high sensitivity functional thermal infrared imaging (fTII).

As outlined in Chapter 2, thermal imaging is an upcoming physiological technique that allows recordings of cutaneous temperature variations wirelessly, without interfering with the experimental procedure or the participant's biological movements (Pavlidis, Tsiamyrtzis, Shastri, Wesley, Zhou, et al. 2012). Physiological observations of an affective nature are primarily related to subcutaneous vasoconstriction or vasodilation as well as heart rate and blood flow (Kistler, Mariazouls, Berlepsch, 1998). The validity of this technique for the measurement of various types of arousal has been demonstrated by simultaneous recording of proven measures such as GSR and Laser Doppler flowmetry (Pavlidis et al., 2012; Kistler, et al., 1998).

The effects of social attention (direct gaze/averted-head-gaze) and proximity (social space – 4 meters/intimate space – 0.5 meters) on facial temperature have been examined. While the majority of studies measuring facial temperature have so far only looked at one site, in the current experiment multiple sites have been examined in order to get a more accurate index of temperature fluctuations in the face as a function of condition. Temperature was measured from six regions of interest on the face selected on the basis of previous research: 1) the nose (Ioannou, et al., 2013; Nakayam, et al., 2005; Kuraoka, Nakamura, 2011); 2) chin; 3) cheeks (Nakanishi, Matsumura, 2008); 4) periorbital region (Hahn, et al., 2012; Pavlidis, et al., 2002; Pavlidis, et al., 2001); 5) maxillary area (Shastri, et al., 2012); 6) forehead (Zhu, et al., 2008). It is expected that the highest values of physiological arousal are going to be observed when the experimenter looks at the participant from intimate compared to social distance. In addition, being in social space should produce a greater effect when the experimenter's gaze is directed towards the participant, than when averted. Finally, the experimenter being in intimate space and not looking at the participant should have a greater effect than when the experimenter will be in social space, independently of gaze direction.

5.2 Methods

Ethics

The Research Ethics committee of the Faculty of Science of the University of Portsmouth gave approval for the study. Experimental procedures were in line with the declaration of Helsinki and the code of human research ethics of the British Psychological society.

Participants

Eighteen female participants were recruited for the study with an age range of 19-21 years old (*M*= 19.83, *SD* = 1.30). Exclusion criteria for participation in the study included gender (males), neurological or mental illness, as well as psychophysiological disorders. In order to improve the reliability of the physiological observations, consumption of vasoactive substances (nicotine, caffeine, alcohol) for at least 2-3 hours prior of participation was prohibited. Female participants came from a range of cultural backgrounds and recruitment was performed through personal contacts and the University of Portsmouth recruitment database.

Design

A 2 x 2 x 2 mixed factorial design was employed. The within subjects factors were gaze (direct gaze vs. averted gaze) and distance (social space vs. intimate space). The independent groups factor was order (intimate space then social space vs. social space then intimate space). The order of gaze vs. gaze aversion was fixed with the gaze aversion condition always occurring first. The dependent variable examined was face skin temperature on six sites on the face.

Procedure

Upon arrival participants completed an Informed Consent Form, and then the BIS/BAS questionnaire (White, Carver, 1994). They were then escorted to the test laboratory where they were instructed to sit comfortably on a chair. Prior to the start of the experimental procedure, the participants were familiarized with a buzzer that was an integral part of the experimental protocol. During this period they were exposed to the sound of the buzzer, held the buzzer as it produced the sound and were fully informed about the reason why a buzzer would be used. Prior to any recordings the participants spent at least 10-15 minutes in the test laboratory. During the experimental procedure the female experimenter moved from intimate (0.5 m) to social space (4m) or from social to intimate space. Visual floor markers were provided to the experimenter to define the precise distance from the participants, as well as other filler floor marks to avoid prediction of the experimental order. The transition from one experimental phase to another was signaled every 40 s by the buzzer. The buzzer sounded six times signaling: a) the start of the experiment; b) the second experimental phase; c) the transition period from one social space to another; d) the third experimental phase; e) the final phase; f) the end of the experiment. Once the experiment was completed participants were given a self-report questionnaire regarding how uncomfortable or comfortable they found the four experimental conditions.

Materials and data acquisition

Data Acquisition

To perform recordings of skin temperature a digital Guide Infrared TP8 camera (ThermoPro™) was used with an uncooled FPA microbolometer (384 × 288 pixels). TP8 provides temperature sensitivity of 0.08 K with an accuracy of $\pm 1\ ^{O}C$ and a sampling rate of 1 frame per second. Prior to recording the camera was placed 50 cm away from the participant, was automatically calibrated and manually fixated on the individual’s face. The sampling rate was set at 50 Hz. The experimental room was set at normal temperature of 20-21 ^{O}C, 60-65% humidity, and with no direct sunlight, ventilation or airflow. Prior to the experimental procedure participants were left for 15 minutes in the experimental room to acclimatize. All experimental recordings took place in the afternoon between 2-4 p.m. In addition, behavioral recordings took place with a frame rate of 50 Hz, by two radio-controlled cameras, both connected to a DVD recorder. The two video signals were combined using a Pinnacle system providing a two-split movie.

Questionnaires.

To control for any personality variables that might have affected autonomic arousal (Critchley, et al., 2001; Gaynor, Baird, 2007; Hughes, et al., 2011) the Behavioral Inhibition System (BIS)/ Behavioral Approach System (BAS) scale (Carver, White, 1994) was administered. For the current study a two-factor model of the BIS scale was used as suggested by Heyms, et al. (2008) where BIS is separated into BIS-anxiety, (4 items) related to conflicts, negative criticism etc. and the freeze/fight/flight system (FFFS-fear,) that relates to fear responsiveness to punishment (3 items). The BAS scale is divided into three subscales: a) Drive for achieving goals (DR-4 items); b) Fun-Seeking or Sensation seeking (FS-4 items, $\alpha = .73$); c) Reward Responsiveness (RR-5 items). For the current study Chronbach's alpha value for BIS-anxiety was .57, for FFFS-fear .63, DR .71, FS .46, and RR .64. The relatively low Chronbach alpha values obtained may be due to the small number of items included in each sub-scale. Nevertheless, this psychometric scale is widely used, has good psychometric properties as well as good convergent and discriminant validity (Campbell-Sills, et al., 2004). Furthermore, four questions were given to the participants regarding pleasantness or unpleasantness of their experience. Rating was provided on a five point Likert-scale ranging from 1 = not uncomfortable to 5 = very uncomfortable. The questions were the following: a) How uncomfortable/comfortable did you feel when the experimenter was looking at you from the back of the room? b) How uncomfortable/comfortable did you feel when the experimenter was looking at you from a close distance? c) How uncomfortable/comfortable did you feel when the experimenter was **not** looking at you from the back of the room? d) How uncomfortable/comfortable did you feel when the experimenter was **not** looking at you from a close distance?

Thermal Data Analyses

Prior to the analyses the behavioral and thermal videos were synchronized in order to represent the same frame in time. Frames were extracted every 5 s using Launch

GuideIR analyzer by Wuhan Infrared Technology (http://www.guide-infrared.com). This was performed in a consistent manner across frames since participants' movements were minimal because of the nature of the experiment. For temperature extraction of the regions of interest (ROI), different shapes were used as indicated by Ioannou, et al., (2014). Circular shapes were used for the nasal tip, cheek, and the peri-orbital regions, whereas rectangular shapes were used for the maxillary area and forehead. Oval shapes were used only for the chin. The shapes did not vary in size across frames and temperature was extracted only when the face was in direct angle to the camera as it has been previously suggested that the above factors induce relative noise (Ioannou et al, 2013). On average 37 frames were extracted for each participant, approximately nine for each phase. The Statistical Package for the Social Sciences, version 17 (SPSS, Chicago, IL) was used to perform the analyses. Data was screened to ensure it was suitable for parametric analysis. A reliability check was conducted. Results from five participants were analyzed by a second rater naïve to the purpose of the study. The second rater performed temperature extraction on the same frames that were primarily selected for the five individuals (x 37 frames). In addition, before moving into a Kappa measure of agreement, the degree of temperature change from one condition to the other was calculated for both coders (see Table 1). Kappa's alphas (*p* <. 05) ranged from moderate .64 (Cheek), to good .70 (Forehead, Periorbital), to very good agreement > .8 (Nose, Chin, Maxillary). To eliminate the possibility that the results from the two raters were different, a 2 x 5 between groups' analysis of variance was conducted. No significant difference was observed between the two groups (*p* <. 05).

5.3. Results

Correlations between temperatures on the six sites on the face

We correlated the temperature values from each site on the face with all other sites on the face for each condition. There were six sites on the face, which gives 15 correlations when all sites are correlated with all other sites. As there were four conditions, this gives a total of sixty correlations. All of the sixty correlations were significant as were the means of the correlations for each condition (intimate space, gaze aversion *M* = .65, *SD* = .13; intimate space, gaze *M* = .67, *SD* = .13; social space, gaze aversion, *M* = .71, *SD* = .12; social space, gaze, *M* = .66, *SD* = .12). This is strong evidence that the different sites on the face are measuring a similar underling construct.

Facial Temperature analyses

To obtain a clearer and more robust pattern on the effects that interpersonal distance and gaze had on facial skin temperature, all regions of interest were averaged (see Table 2, Figure 1) and a 2 x 2 x 2 mixed factorial ANOVA was performed on the averaged data. No significant interaction effects were observed between interpersonal distance, gaze and order, Wilks' Lambda = .96, *F* (1, 16) = .66, *p* = .429, η_p^2 = .04 or order and gaze, Wilks' Lambda = .80, *F* (1, 16) = 3.89, *p* = .066, η_p^2 = .19. There was a significant interaction between interpersonal distance and order, Wilks' Lambda = .41, *F* (1, 16) = 22.68, *p* =.000, η_p^2 = .58 (see Figure 2.). As shown in figure 2 it seems that the interaction is a function of the fact that temperature increases when the experimenter moves from social space to intimate space, but is relatively unaffected by distance when moving from intimate space to social space. This interpretation is supported by simple main effects analyses (with Sidak adjustment). It was observed that there was a significant increase in temperature when the experimenter moved from social space (*M* = 33.20, *SD* = 1.05) to intimate space (*M* = 33.62, *SD* = 1.01), *p* = .000. However, no significant difference was observed in temperature when the experimenter moved from intimate space (*M* = 34.25, *SD* = 1.22) to social space (*M* = 34.32, *SD* = 1.18), *p* = .054 (see also Table 3). There was also an interpersonal distance and gaze interaction, Wilks' Lambda = .76, *F* (1, 16) = 5.03, *p* = .039, η_p^2 = .24 (Figure 3). From figure 3 it appears that the interaction is the result of the fact that the impact of direct gaze on temperature increase is greater in the intimate space condition than in the social space condition. Simple main effects analyses provide some limited support to this interpretation, as there was a significantly higher temperature in the intimate space, direct gaze condition (*M* = 34.02, *SD* = 1.14) than in the intimate space gaze aversion condition (*M* = 33.84, *SD* = 1.75), *p* = .000. The temperature was also significantly higher in the social space condition when the experimenter engaged in direct (*M* = 33.79, *SD* = 1.21) compared to averted gaze (*M* = 33.72, *SD* = 1.29) *p* = .014. However, the difference was greater in the intimate space condition (see also Table 3). There was a significant main effect of gaze with direct gaze having a higher temperature (*M* = 33.90, *SD* = 1.21) than gaze aversion (M = 33.78 SD = 1.16), Wilks' Lambda = .36, *F* (1, 16) = 28.35, *p* = .000, η_p^2 = .64, with a large effect size.

Given the ordinal interaction between gaze and distance it is safe to interpret this main effect by concluding that direct gaze always produces a large effect on facial temperature. There was also a significant effect of interpersonal distance with temperature being higher in the intimate space condition (M = 33.93 SD = 1.15) than in the social space condition (*M* = 33.76, *SD* = 1.23) Wilks' Lambda = .58, *F* (1, 16) = 11.66, *p* = .004, η_p^2 = .42 with a large effect size. Given the interaction results we can again be relatively confident that there is a pervasive and robust elevation of temperature in intimate space.

Individual Region Analyses

The mean values for each of the six regions of interest for each condition were calculated from all 18 individuals and mixed 2 x 2 x 2 factorial ANOVAs were performed on the data for each region of interest. The repeated measures factors were eye contact vs. head gaze aversion; proximity (social space – 4 meters vs. intimate space – 0.5 meters); and the independent groups factor was order (social space then intimate spaces vs. intimate space then social space). On all conditions the three-way interaction was not significant. However, two-way interaction effects were observed between interpersonal distance and order for four out of six regions of interest, and in one region of interest a significant interaction for distance and gaze was found. In the 'normal' order the pattern was very consistent. Temperature increased in all regions of interest when the experimenter moved from social space to intimate space. However, in the 'odd' order the change in temperature was much less consistent. Main effects were observed for distance and gaze in four out of six regions of interest. Temperature was higher in intimate rather than social space and higher in direct gaze compared to averted gaze. However, this latter effect could only be observed within the same interpersonal space (e.g. Intimate Space-Averted Gaze vs. Intimate Space-Direct Gaze). There was no significant main effect of order.

Questionnaires analyses.

There was no significant correlation between BIS/BASS scores and temperatures on the six regions of interest. A one way ANOVA was conducted to examine the effect of the four experimental conditions (gaze aversion, intimate space, gaze aversion, social space, gaze intimate space, gaze social space) on the subjective pleasantness rating scores.

Results showed that unpleasantness scores to the four subjective questions were significantly different *F* (3, 68) = 10.10, *p* = .000, η_p^2 = .3 with a medium effect size. Post-hoc comparisons using Tukey HSD indicated that unpleasantness scores were significantly higher when the experimenter was in intimate space and engaged in direct gaze (*M* = 4.17, *SD* = 1.1) compared to direct gaze in social space (*M* = 3.06, *SD* = .87), averted gaze in intimate space (*M* = 2.89, *SD* = 1.18) and averted gaze in social space (*M* = 2.89, *SD* = 1.18). No other significant differences between groups were observed.

Visual Inspection of the Thermograms

Four images were created from two randomly selected individuals for each experimental order (Figure 4). The images were taken 10 seconds prior to the end of each phase. This was performed in order to allow enough time for large temperature effects to take place on the skin surface that would enable a vibrant visual assessment of the infrared image. Moving from social space to intimate space, it can clearly be seen that in some regions of the face temperature changes are more clear-cut than in others. Temperature of the nose is making more evident linear increases when the experimenter is moving from social to intimate space. This can be seen by the color of the nose that gradually changes from dark blue to green. In the second sequence, however, when moving from intimate to social space a less clear picture can be seen. Hardly any temperature changes take place in intimate distance, independent of the experimenter gaze. The same temperature effect persists also in interpersonal social space regardless on gaze. However if the first and third images (of the second order) are observed closely, a small yellow patch between the eyebrows seems to disappear in the second and fourth image of the figure. Moreover, while moving from social space into intimate space the color of the forehead changes from shades of red to bright pink, with larger increments in temperature in isolated areas that are innervated by supraorbital vessels. Furthermore, the cheeks are changing color from green to red, whereas in the second order this is not so evident. Small temperature increments from green to yellow are observed from the first to the second image, changing from yellow to red, particularly near the ear. The same can be observed when looking at the third and forth images. The peri-orbital region in the first row (normal order) seems to increase in temperature as the red color is substituted by white, however changes are small. In the second row no visual changes are observed. The maxillary area shows a linear increase in temperature moving from social space to intimate space. However, in the case of moving from intimate to social space an increase of temperature is observed from averted gaze to directed gaze, particularly when moving from intimate to social space. Finally, the chin seems to increase in temperature when the experimenter moves from social to intimate space. However, temperature seems to remain rather stable when in social space independently from gaze. During the opposite sequence, temperature seems to remain stable. However, by inspecting closer the chin an increase is observed in the number of red dots, particularly when in social space from averted to directed gaze. The same is observed between the third and fourth image, although to a lesser extent. The visual results observed in the second condition (Intimate → social) represent an overcompensation effect where temperatures started off higher and decreased less. In addition, facial regions may not have had the temporal opportunity to show a temperature change from one condition to the other, as only a 40 s time interval was provided. These results may show a physiological temperature “spill over” effect from the most arousing condition to the next as can happen during the transition from intimate to social space.

5.4. Discussion

In the present study we explicitly modulated the social context in which gaze and proximity occurred by having two different experimental sequences. One sequence involved what would be considered a socially normal shift from a social distance to an intimate distance. The other involved a socially odd shift from an intimate distance to a social distance. At each distance there was a fixed sequence of the two gaze conditions: direct gaze always followed averted gaze. Analyses showed that when moving from social to intimate distance, facial temperature rose on average by .42 °C. However, no significant temperature change between the two distances was observed when the socially odd sequence took place. On the other hand, the effects of direct gaze compared to averted gaze were significant, independent of order: direct gaze led to a higher temperature than averted gaze at both the intimate distance (a difference of .17 C) and at the social distance (a difference of .10 C). The subjective ratings given by participants on the self-report unpleasantness scales supported the thermal findings: the highest ‘uncomfortable’ scores were obtained by direct gaze in intimate and social distance, followed by averted gaze in intimate and social distance.

Previous studies suggest that gaze and distance seem to have a consistently robust effect on a range of psychophysiological measures. The current findings obtained using fTII are in agreement with results obtained with GSR (McBride, et al., 1965; Nichols, Champness, 1971) and electroencephalography (Gale, et al., 1975). As previously observed, direct gaze not only increases arousal but also seems to be mediated in intensity by interpersonal distance. Although in the present study participants could not alter their interpersonal distance, the findings provide support for Argyle and Dean's (1965) equilibrium theory, arguing that interpersonal distance and gaze interact to modulate arousal. In the present study, as interpersonal distance decreased, the arousal effects of direct gaze were greater. Overall it would appear that interpersonal distance and gaze interact to have strong effects on human physiology, with temperature variability of the face being differently affected by each experimental condition. These bodily signs of autonomic arousal picked up by thermal imaging reveal the preparedness of the organism to support behavioral engagement strategies, whether these involve social interaction or mechanisms of avoidance (Bodenhauzen, Hugenberg, 2009; Porges, 2001).

The increase in facial temperature creates a "physiological paradox". Although the overall subjective experience of personal space intrusion as well as eye contact independent of distance was rated as uncomfortable, there was a rise rather than a dip in facial temperature. Previous literature using thermal imaging has found that negative emotions such as fear (Kistler et al., 1998; Nakayam, et al., 2005; Kuraoka, Nakamura, 2011), stress (Pavlides et al., 2012), and guilt (Ioannou et al., 2013), lead to a drop in the temperature of the nose, the maxillary area, the forehead, as well as the fingers, as a result of peripheral vasoconstriction. From the present results it seems that the experience of interpersonal proximity and gaze does not fall physiologically into the same category. Increases in facial temperature have been observed in experiments of social contact (Hahn et al, (2012) and anxiety (Pavlides et al., 2002; Tsiamyrtzis et al., 2006; Zhu et al., 2008). In the case of Hahn et al., (2012) participants were touched on various parts of the body by female and male experimenters using a handheld light-flashing device. Body parts that were touched were the face and chest (high-intimate) and the arm and palm (low intimate). What was observed was that when participants were touched on high intimate areas temperature increased, with an even greater increase when this act was performed by an experimenter of the opposite sex. These increases in temperature were localized on the nose, lip and peri-orbital regions of the face. Anxiety in individuals seems to cause a similar effect on facial temperature. Participants who were interrogated for a mock crime that they had just committed and who tried to defend their innocence showed an increase in temperature near the peri-orbital (Tsiamyrtzis et al., 2006) and the supraorbital vessels of the forehead (Pavlides et al., 2002; Zhu et al., 2007). The results obtained by Pavlides et al., (2002) are consistent with traditional polygraphs tests that use physiological measures of pulse, blood pressure, perspiration and skin conductivity to draw conclusion about the honesty of the individual. Behaviorally, something common among the above mentioned experiments is the challenging nature of the social situation. Pavlidis and Levine (2002) suggested that temperature increase results from increased blood perfusion to the surface of the skin. Increased blood perfusion is the result of increased delivery of blood to body tissue and the heart is the organ of the body that can sustain such a function (Kreibig, 2010). Thus, judging from the previous literature on thermal imaging, increased blood flow to the surface of the skin is the result of increased heart rate that causes the skin temperature to rise.

The present physiological findings as well as the observation made by previous research are in support of Polyvagal theory (Porges, 2001). According to this theory mammalian evolution favored the development of an efficient neural control model, which provided increased control of the heart via the myelinated vagus, the 10th cranial nerve. When needed, sympathetic tone expression and increased cardiac output support – by means of vagal disengagement – transitory mobilization states without activating the costly sympathetic or adrenal system. Furthermore, the temperature rise observed in the present study is not in agreement with Bell et al., (1996) as the proposed "stress theory" should also be related to temperature decrease (Pavlides et al., 2012; Ioannou et al., 2013; Kistler et al., 1998).

No significant order effect was observed. In the "odd" sequence what both Figure 1 and Figure 4 show is that no significant temperature change took place from one condition to the other and only minor temperature changes were observed. Approaching the individual initially from intimate distance and then moving to social space yielded no significant temperature changes. Although at the group level significant results were obtained in direct gaze compared to averted gaze, independently from sequence, this outcome might have not reached statistical significance because of the power of the normal experimental order (Social distance → Intimate distance). The way the experiment was designed seems to favor the approach moving from social distance to intimate distance, as a linear increase in temperature was observed. In this order physiological effects seem to intensify from one condition to the other. On the other hand, moving from intimate to social distance, temperature changes did not behave in the same manner. What can be observed during the ‘odd’ experimental sequence is an overcompensation effect and a physiological “spill-over” from the most arousing condition to the least arousing one. Although in intimate distance an increase in temperature from averted to direct gaze was observed, no temperature change took place during the transition phase from intimate to social distance. Facial temperature likely did not have the opportunity in such a short time to reject the physiological changes that took place at intimate distance along with direct gaze before moving to social distance and averted gaze.

Literature on thermal imaging does not provide evidence on the time needed for facial temperature to return back to baseline or rest values when the facial temperature rises. However, temporal evidence exists on how temperature behaves when temperature decreases. Nakayama et al. (2005) reported that after stimulation, 220-280 s are needed in order for temperature of the nose to return to baseline values. Moreover Kuraoka and Nakamura (2011) reported that changes on the nose lasted on average for 60 s before descending back to baseline values. Evidence from rodents suggests that according to the region of interest there is also the appropriate expected delay (Vianna, Carrive 2005). The back, head and the body of the animal took approximately 60-75 minutes to return to baseline, whereas the eyes, tails and paws 14, 10 and 15 minutes, respectively. Although changes in heart rate take place much more rapidly than changes in vasoconstriction (Kisler et al., 1998, Vianna, Carrive, 2005) and despite the fact that the two physiological phenomena have different underlying mechanisms, we believe that in the present study there was not enough time in the transition from intimate to social distance for adequate heat changes to be observed between conditions. Finally, although temperature changes in the direction of the physiological excitation can be rapidly observed within 15-20 s (Nakayama et al., 2005; Kistler et al., 1998; Kuraoka, Nakamura, 2011), as a result of an affective stimulus, temperature restoration takes substantially longer.

The present study demonstrates that this novel, wireless, physiological technique has the sensitivity not only for picking up changes in the intensity of the stimulus but also in replicating results observed by previous studies using other widely established physiological measures. Through this experimental model the foundation stone has been set for other studies in the clinical domain, whether this relates to diagnoses or the efficacy of treatment (Yehuda et al., 1996; Jansen et al., 2000; De Bellis et al., 1994; Dalton et al., 2005; Horley, Williams, Gonsalvez, & Gordon, 2002). Thermal imaging is a valuable tool in studies in which participants cannot express their emotions verbally (Uithol, Paulus, 2013; Kyselo, Di Paolo, 2013; Nakanishi & Matsumura, 2008) or in studies where emotional arousal can only be inferred by coding behavior and by measuring physiological responses (Vianna & Carrive 2005).

Functional thermal imaging has the potential for identifying subtle psychophysiological changes taking place on the surface of the skin as a result of underlying vasoconstriction or heart rate variability. Although in the present study a rise in temperature was observed, no other physiological measures were obtained to clearly explain why such changes took place. Literature on the topic provides some evidence as to why this might have happened. On the basis of the findings from the literature (Pavlidis & Levine, 2002) we can speculate that this could be related to increased heart rate output. It would be important in future studies investigating temperature changes to employ heart measures to explain temperature-related physiological observations. In addition, related to the context of the current study are the other two distances “personal” and “public” which would also be nice to investigate (Hall 1966). Furthermore, since in the current experiment female participants were exposed to female experimenters, mixed gender dyads could be added as well as mixed gender groups. Finally, thermal imaging has a poor temporal latency despite its sensitivity in picking up small fluctuations in temperature. This is not because of the inadequacy of the technique but because of the temporal latency that the skin needs to exhibit changes of physiological nature, whether these are the results of vascular constriction or of increased blood flow. Thus it is important to leave adequate time from one condition to the next in order for temperature to return approximately to baseline values.

Conclusions

Interpersonal distance and perceived gaze are two related social constructs with each one imposing its presence on the physiological reactions of the receiver. Current observations of these phenomena suggest that direct gaze compared to averted gaze affects autonomic reactions and facial temperature. These results persist independently of the distance from which gaze occurred. In terms of interpersonal distance, intruding an individual's intimate space leads to a marked increase in temperature. This result however is only evident when there is an approach from social to intimate distance. On the other hand, a difference in temperature is not observed when the individual is primarily approached by the experimenter in intimate distance who then moves to social distance. This phenomenon of "physiological spill-over" represents an effect lasting longer than the pre-defined time interval of the experimental phase. Skin temperature does not recover after being exposed to the most arousing intimate condition and this effect lasts after transition is made to the least arousing condition in social space. Despite the methodological significance of the study, gaze and interpersonal distance have their own piece of the pie to claim in social interaction. Physiological reactions obtained by facial skin temperature suggest that preparatory action for engagement or avoidance takes place when gaze is engaged and when intimate space is violated. However, in humans social elements of space and gaze are not treated as threatening since, if they were, a drop in temperature showing the full blown effects of threat would have been observed. These results rather suggest a physiological preparatory action by the organism for what will follow whether this is an attack or a pleasant social interaction.

Tables & Figures

Table 1. The degree of temperature change from one condition to another based on the coding of two independent ratters.

NoseR1_R2		**Maxil.R1_R2**		**Per.R1_R2**		**ChinR1_R2**		**For.R1_R2**		**Cheek.R1_R2**	
.30	.40	.30	.30	.10	.10	.10	.10	.20	.30	.20	.20
-.50	-.50	-.10	-.10	-.10	-.10	.10	.10	-.10	-.10	.10	.10
.10	.10	.10	.10	-.10	-.10	-.10	-.10	-.80	-.80	.50	.50
.20	.20	.40	.50	.40	.40	.40	.40	.50	.60	.50	.60
.40	.40	-.70	-.70	.70	.70	.20	.20	.30	.40	.70	.70
.30	.30	.20	.20	.10	.00	.40	.40	.30	.30	.70	.80
.50	.50	.50	.50	.30	.40	.20	.20	.30	.30	.70	.80
-.10	-.10	-.10	.00	.80	.80	.30	.30	.60	.50	-.10	-.10
-.30	-.30	-.30	-.30	.80	.90	-.20	-.20	-.20	-.20	-.50	-.50
1.00	1.00	.80	.80	-.70	-.70	.30	.30	.60	.60	.40	.40
-.10	-.10	.40	.40	.30	.30	.10	.10	.20	.20	.20	.30
-.30	-.40	-.20	-.20	-.20	-.20	.40	.40	-.10	-.10	-.70	-.80
.00	.00	-.30	-.30	.90	1.30	.00	.00	-.40	-.40	.30	.30
.00	.00	-.10	-.10	.00	.00	.00	.00	-.40	-.40	.00	.00
.50	.60	.20	.20	.00	.00	.50	.50	.10	.10	.30	.30

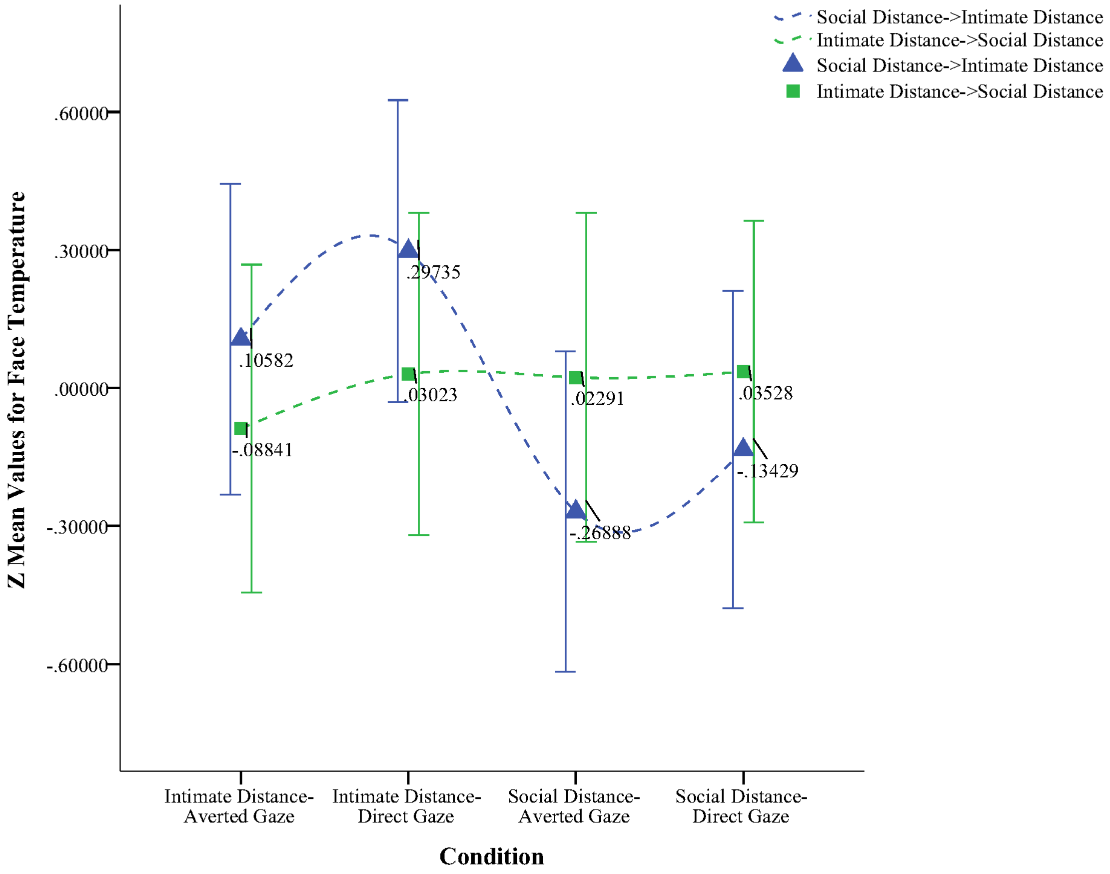
Table 2. Mean Temperature values for the face according to order.

Region	**Condition**	**Order**	***M***	***SD***	***N***
Face	Intimate Space-Averted Gaze	Intimate →Social	34.17	1.26	9
		Social→Intimate	33.52	1.04	9
		Total	33.84	1.17	18
	Intimate Space-Direct Gaze	Intimate →Social	34.31	1.24	9
		Social→Intimate	33.71	1.01	9
		Total	34.02	1.14	18
	Social Space-Averted Gaze	Intimate →Social	34.30	1.26	9
		Social→Intimate	33.13	1.07	9
		Total	33.72	1.29	18
	Social Space-Direct Gaze	Intimate →Social	34.32	1.16	9
		Social →Intimate	33.27	1.06	9
		Total	33.79	1.21	18

Table 3. Results of the simple main effect analyses between distance and order as well as distance and gaze.
Note: Order 1 = Social Space->Intimate Space, Order 2 = Intimate Space -> Social Space

		Mean Diff.	*SE*	*Wilks' Lamda*	*F*	*Hypoth. df.*	*Err. df*	**Sig. (two-tailed)**	η_p^2
Test 1	Intimate Space (Order 1) vs. Social Space (Order 1)	.417	.072	.324	33.44	1	16	.000	.676
	Intimate Space (Order 2) vs. Social Space (Order 2)	-.069	.072	.946	.91	1	16	.354	.054
Test 2	Intimate Space-Eye Contact vs. Intimate Space-Averted Gaze	.169	.034	.389	25.10	1	16	.000	.611
	Social Space-Eye Contact vs. Social Space-Averted Gaze	.077	.028	.676	7.66	1	16	.014	.324

Figure 1. Line graph illustrating the temperature of the face based on the experimental order of the four conditions



Error bars: +/- 1 SE

Figure 2. Line graph representing the interaction effect between interpersonal distance and order.

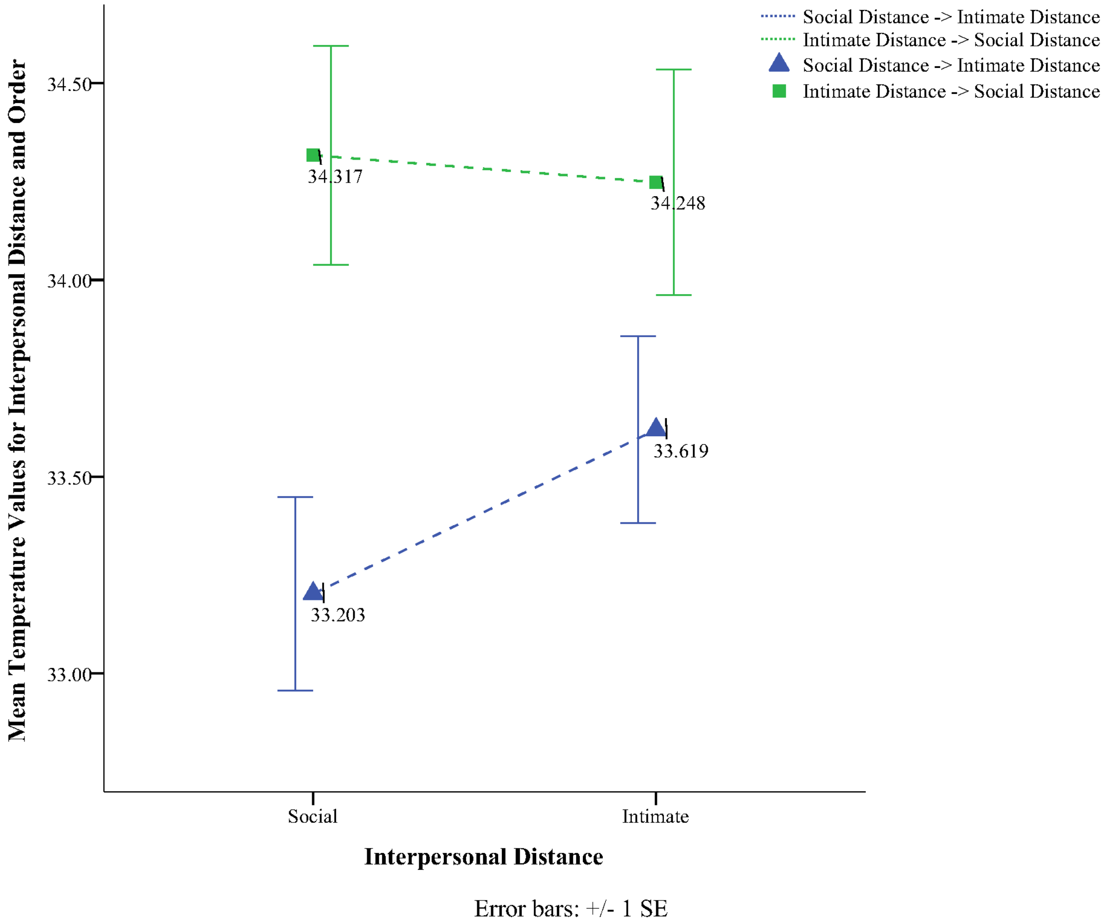


Figure 3. Line graph representing the interaction effect between interpersonal distance and gaze.

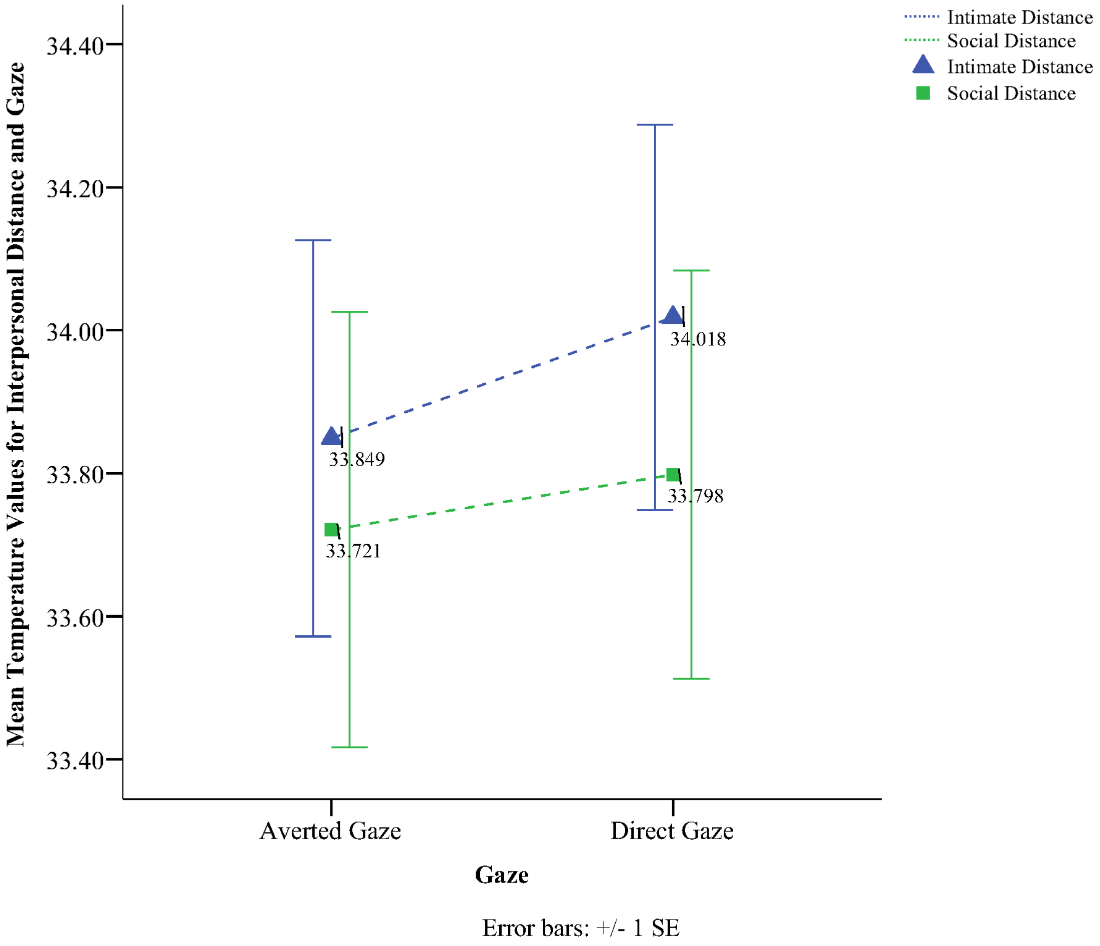
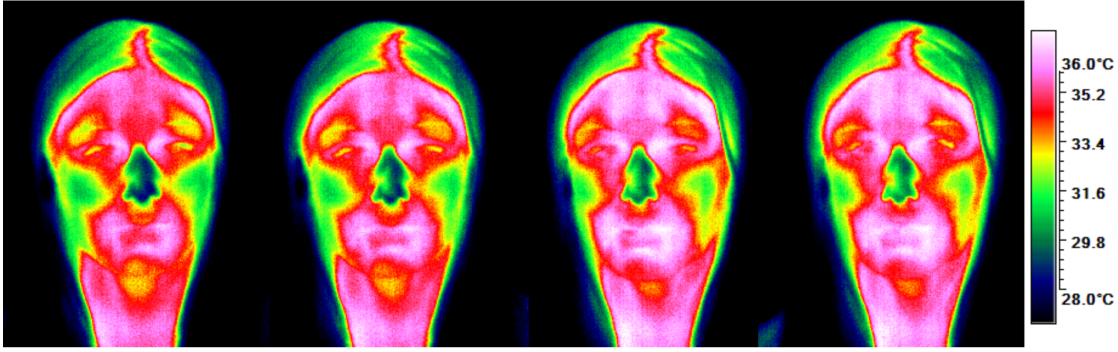
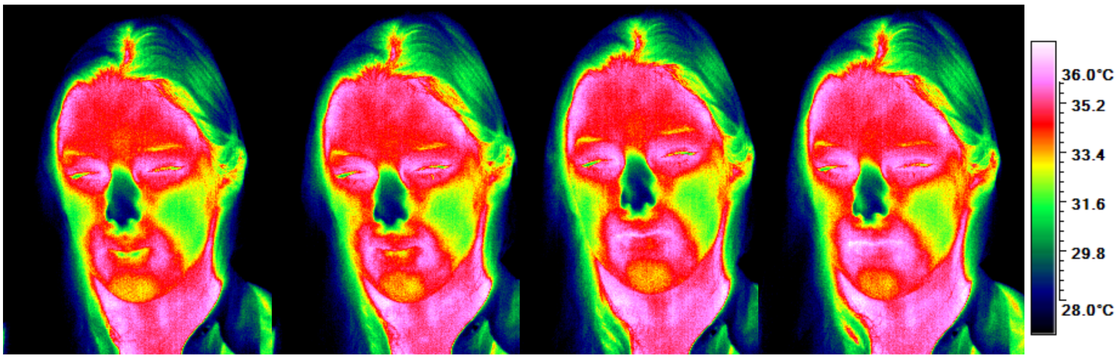


Figure 4. Visual illustration of the development of temperature for each conditions according to order.



Social Space-Averted Gaze Social Space-Direct Gaze Intimate Space-Averted Gaze Intimate Space-Direct Gaze



Intimate Space-Averted Gaze Intimate Space-Direct Gaze Social Space-Averted Gaze Social Space-Direct Gaze

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Chapter 6

6. General Discussion, conclusions and future directions

The present studies have demonstrated facial thermal variation in response to emotionally-related stimuli. Physiological correlates of temperature seem to be shared among conspecifics. Experimental scenarios of distress have shown that the emotional world of the observer and the actor are entangled into one (Ebisch et al., 2013). Facial temperature in both the mother and her child was consistently rising or decreasing on the nose tip and upper lip, depending on the experimental phase. This relationship of emotional sharing seems to be modulated by the level of the family bond as mother-child dyads seems to be in better temporal synchrony than woman-unknown child dyads (Manini, Cardone, Ebisch, Bafunno, Aureli, Merla, 2013). Despite the importance of these findings, little was understood regarding the physiological origin that drives temperature change on the face. In a follow up study the same experimental protocol was used, with a greater sample size and a particular focus on the physiological nature of self-conscious emotions in children. It was observed that the facial region that had the most consistency among participants was the nose-tip, where during distress a decrease in temperature was observed (Ioannou et al., 2013). The forehead, the maxillary area, the peri-orbital region, the chin, and the corrugator muscle did not show any consistency among participants or during the experimental procedure. A possible reason for this phenomenon may be the absence of a true reference point for temperature comparison. To further understand the affective nature of the temperature change of the face and the underpinning physiological mechanisms, a systematic review of the emotional literature in thermal imaging literature was performed (Ioannou, Gallese, & Merla, 2014). As a technique thermal imaging has been applied to the study of fear, empathy, distress, occupational load, anxiety, lie detection, and sexual attraction. Most primary emotions that were examined in a negative framework such as fear, empathy in the context of a mishap, distress, and increased workload have been associated with a temperature decrease on the nose, paws, tail and the maxillary area. These changes have been accounted to the adrenergic system leading to peripheral vascular constriction on the extremities and increase in sweating on the upper lip. On the other hand, emotions that dealt with social contact, sexual interaction, and interrogation showed an increase in temperature on the peri-orbital region, the mouth, the nose and in certain cases on the forehead and corrugator muscles. Empirical evidence ground these changes to the vagal system and to heart rate increase. Others speculated that thermal changes on the peri-orbital regions are the result of rapid eye movement facilitating “fight or flight” responses as a result of demand and supply. Similar reasons have been proposed in the case of the corrugator muscle, where temperature rise has been revealed in response to increased frowning during interrogation. Although the increased muscular activity on both the peri-orbital region and the corrugator muscle may have contributed to some extent to the recorded temperature rise, it is highly likely that the main reason for this change be the blood flow increase in the superior facial vessels by heart rate acceleration. From the literature review it was clear that only few studies have investigated how interpersonal interaction effects physiological temperature variability. To further explore interpersonal factors that may affect the facial thermal print, popular scientific examples in the domain of social psychology were revisited (Argyle and Dean, 1965). By manipulating gaze and interpersonal proximity between the experimenter and the participant it was observed that direct gaze has an effect on facial temperature independently of interpersonal distance. Temperature on all six regions of the face (chin, cheek, peri-orbital, maxillary, nose, forehead) was steadily rising throughout the experimental manipulation. Interpersonal space violation also led to a temperature rise. The results of this study suggest that interpersonal interaction in the form of gaze and interpersonal proximity lead to physiological social calibrations within the autonomic nervous system, adapting to foster the requirements and type of the social interaction at stake (e.g hostile, sexual, friendly) (Ioannou et al, 2014).

Future studies should extend the above mentioned experimental paradigms to the clinical domain by evaluating moral development in the context of guilt (Ioannou et al., 2013), the sympathetic relationship between depressed mothers and their children (Ebisch et al., 2012), or the emotional reactivity of individuals with personality and conduct disorders in an interpersonal experimental setting (Ioannou et al., 2014). Thermal imaging is a promising technique for sensitive population groups and for naturalistic experimental paradigms, as all observations are wireless and do not interfere with the experimental procedure. Nevertheless, thermal imaging as most physiological techniques suffers from a lack of definition of a true baseline in experimental recordings. No evidence exists so far that establishes what to expect on different regions of the face, in terms of baseline temperatures, temperature range and individual variability. This lack of empirical evidence on what constitutes a baseline, poses major methodological challenges to the use and the development of the technique and it should be addressed in the near future.

Moreover, paradoxical as it may seem, no study has to date examined the communicative value of temperature change and how this is reflected in terms of facial coloration. This would be an important advancement for thermal imaging as a technique. It would be important for future studies to collect along with thermal imaging also heart rate measures, in order for more concrete conclusions to be drawn on the autonomic nature of the temperature change. The manual extraction of temperature data is a rather laborious process and reliable automated methods would ease the way in data collection and analysis, saving resources and time. Although certain packages do exist (Shastri et al., 2012) they have not been widely used probably because of methodological flaws.

In conclusion, functional Thermal Infrared Imaging has shown its reliability in collecting physiological data in real life situations. The true strength of this technique lies in the fact that the face (unlike other body parts) is not occluded and temperature provides a blend of physiological information regarding autonomic responses. Facial temperature conveys information on vascular constriction (Ioannou et al., 2013; Vianna & Carrive, 2005; Kistler, Mariazouls, & Berlepsch, 1998; Nakayama, Goto, Kuraoka, Nakamura, 2005), blood flow increase (Ioannou et al., 2014; Pavlidis, Eberhardt, & Levine, 2002) and emotional sweating (Shastri, Merla, Tsiamyrtzis, & Pavlidis, 2009), covering a wide range of emotional responses. Although still in its infancy, functional thermal imaging benefits from the rich physiological literature on emotion (Kreibig, 2010) and it is wise to use it in conjunction with well-established physiological measures of autonomic responses such as heart rate (Berntson, Cacioppo, Quigley, 1993).

For the future of thermal imaging it is important to develop a consistent methodology as well as fITI user-friendly software to be used for temperature extraction and freely available to all users. This will overcome problems across studies related to temperature extraction, such as the size of ROI, where the ROI should be placed, whether the maximum, average, or minimum temperature should be taken into account for data analysis and whether observations should be based on a number of active voxels. Some progress has been made on these matters in terms of software development (Dowdal et al, 2006; Merla Di Donato, Romani, 2002). However, a methodological protocol to be followed by the majority of fITI studies is still lacking. Finally, it would be important to establish a link between thermal observations and brain regions entertaining bidirectional connections with visceral organs (Kuraoka & Nakamura, 2011). Stimulation of the central nucleus (Bagshaw & Coppock, 1968; Laine, Splitler, Mosher & Gothard, 2009) as well as of the corticomedial nucleus (Potegal, Hebert, DeCoster, & Meyerhoff, 1996) of the amygdala provide good cortical candidates for observing thermal changes associated with negative emotional states. Electroencephalography can also be integrated with thermal imaging since it does not occlude the participants face, thus allowing the associations of subcortical emotion-related regions with the thermal print.

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