The effect of pleasant touch on nose skin temperature, heart rate and heart rate variability: preliminary results in a male laboratory rhesus monkey

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Human and animal studies emphasize the importance of affiliative touch among conspecifics, both from the behavioral and physiological perspectives. Among non-human primates, allogrooming, and in particular the pleasant sweeping motion occurring during it, could be considered analogous to human social affiliative touch. Despite the evidences of the effects of affiliative touch in terms of heart rate (HR) and heart rate variability (HRV), both in humans and non-human primates, the physiological consequences have never been investigated in respect to the body temperature changes through infrared thermography (IRT). The aim of the present study was to investigate for the first time in a male rhesus monkey, the physiological effects of sweeping the back at different speeds in terms of nose skin temperature changes, and to explore the possible relationship with the HR and HRV. The preliminary results underline that sweeping the back at a speed of 5–10 cm/sec determined an increment of the nose skin temperature and HRV, together with a decrement of the HR. These preliminary data represent the first evidence of the body temperature changes manifesting during affiliative touch at the speed of 5–10 cm/sec in non-human primates and the existence of a possible relationship among the body temperature, HR and HRV. This study represents an important starting point in order to investigate the affiliative pleasant social touch by means of non-invasive techniques (*e.g.* the IRT), and to deeply examine the correlation between body temperature and cardiac changes, both in humans and non-human primates.

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Abstract

Human and animal studies emphasize the importance of affiliative touch among conspecifics, both from the behavioral and physiological perspectives. Among non-human primates, allogrooming, and in particular the pleasant sweeping motion occurring during it, could be considered analogous to human social affiliative touch. Despite the evidences of the effects of affiliative touch in terms of heart rate (HR) and heart rate variability (HRV), both in humans and non-human primates, the physiological consequences have never been investigated in respect to the body temperature changes through infrared thermography (IRT). The aim of the present study was to investigate for the first time in a male rhesus monkey, the physiological effects of sweeping the back at different speeds in terms of nose skin temperature changes, and to explore the possible relationship with the HR and HRV. The preliminary results underline that sweeping the back at a speed of 5–10 cm/sec determined an increment of the nose skin temperature and HRV, together with a decrement of the HR. These preliminary data represent the first evidence of the body temperature changes manifesting during affiliative touch at the speed of 5–10 cm/sec in non-human primates and the existence of a possible relationship among the body temperature, HR and HRV. This study represents an important starting point in order to investigate the affiliative pleasant social touch by means of non-invasive techniques (*e.g.* the IRT), and to deeply examine the correlation between body temperature and cardiac changes, both in humans and non-human primates.

1. Introduction

Numerous human studies have investigated social skin-to-skin affiliative touch, finding that a caress given at a speed of 1–10 cm/sec 1) activated the C-tactile fibers (CT fibers) at the level of the hairy skin (Loken et al., 2009; Liljencrantz et al., 2014); 2) determined a decrement of heart rate (HR) and increment of heart rate variability (HRV), *i.e.* a positive modulation of the vagal tone (Porges, 2003; Porges, 2007; Lindgren et al., 2010); and 3) activated the insular cortex at the central nervous system level, where the emotional codification of the peripheral sensory stimuli occur (Olausson et al., 2002; Olauddon et al., 2008). As with caress in humans, allogrooming among non-human primates may be considered as social affiliative touch. Allogrooming is a complex behavior, characterized by the bimanual actions of two motions that are repeated consecutively and rhythmically: the sweep and the pick of the fur with the fingernails in a precision grip. Concerning the autonomic consequences it was reported that when received, allogrooming determined a decrement of the HR (Boccia, 1989; Aureli, Preston & de Waal, 1999), and a reduction of the cortisol levels during both passive (Gust et al., 1993) and active grooming (Shutt et al., 2007). Furthermore, the cardio-physiological effects of picking and sweeping separately, in laboratory non-human primates were recently investigated. The studies highlighted that both human picking (Grandi & Ishida, 2015) and sweeping (Grandi, Rodà & Ishida, 2015) determined the increment of HRV and decrement of HR, even in a typical experimental environment. In particular, the sweep performed on the back of a male rhesus monkey with speeds of 5 cm/sec and 10 cm/sec determined a decrement of the HR and increment of HRV. Lower and higher speeds resulted in opposing cardio-physiological effects. Moreover, in the latter study (Grandi, Rodà & Ishida, 2015), a preliminary kinematic analysis underscored that the speed of the real sweeping movement of allogrooming among free ranging monkeys was within the optimal speed range to activate the CT fibers in humans (*i.e.* 9.31 cm/sec). These results strongly support Dunbar's (Dunbar, 2010) hypothesis concerning the role of CT-fibers during the sweeping motion of allogrooming, and consequently its meaning as affiliative touch, as human caress.

The physiological effects of affiliative touch have never been investigated in respect to body temperature changes, either in human or in non-human primates. Utilizing infrared thermography (IRT) to measure the energy radiating from the subject and translate it into a temperature value is a useful non-invasive technique to facilitate study of both the positive and negative emotions in humans (see ref. Ioannou, Gallese & Merla, 2014 for an exhaustive review) and animals (Stewart et al., 2007; Travain et al., 2015). Nevertheless, the literature associated with non-human primates in the field of thermography is particularly poor. The first studies were conducted in 2005 by Nakayama and colleagues (Nakayama et al., 2005), and in 2011 by Kuraoka and colleagues (Kuraoka & Nakamura, 2011), before the final one performed by Ioannou and colleagues in 2015 (Ioannou, Chotard & Davila-Ross, 2015). In the first two experiments, the monkeys were exposed to negative stimuli and a decrement of the nose temperature was demonstrated; while in the last study, the increment of the nose temperature of monkeys during a positive-related situation (*e.g.* during play) was evidenced. From these findings we may conclude that the nose temperature of monkeys increases when the animal is exposed to a positive situation, and decreases if the situation then assumes a negative value.

The aim of the present study was to investigate for the first time in non-human primates, the effect of sweeping the back in terms of nose skin temperature change. The preliminary results presented here represent the first evidence of changes in the peripheral body temperature during affiliative touch at the speed of 5–10 cm/sec in an experimental male rhesus monkey, together with a potential relationship among body temperature, HR and HRV.

2. Material and Methods

2.1 Subject

We performed electrocardiogram (ECG) and IRT recordings of one experimental male rhesus monkey (*Macaca mulatta*) aged five years and weighing 5.6 Kg. The monkey had participated in a neurophysiological experiment for the last two years and two ECG recordings, prior to the present experiment. The monkey was trained and habituated to a custom-designed primate chair. All experimental protocols were approved by the Veterinarian Animal Care and Use Committee of the University of Parma, and complied with European law on the humane care and use of laboratory animals. The monkey was kept in an individual primate cage (Tecniplast S.p.A, Buguggiate, Italy; approximately 180 cm high, 90 cm wide, 120 cm deep) in an air-conditioned room maintained at a consistent temperature of 25–26° C. The monkey had access to toys, mirrors and swings in his own cage, together with visual, auditory and olfactory contact with other monkeys and physical contact with neighboring monkeys. The well-being and health conditions of the monkeys were consistently monitored by the institutional veterinary doctor of the University of Parma (Italy).

2.2 Recording Procedure

The measurements were conducted inside the laboratory and prior to the morning feed. The laboratory was a familiar environment for the monkey since he had participated in previous studies. Before each session, the monkey was seated in the primate chair and then brought to the laboratory; then 1) the head was fixed with the head holder, and 2) the ECG electrodes were attached to the back of the monkey. As the position of the monkey in the laboratory was fixed it was therefore possible to position the IRT camera in order for it to be correctly located in front of the monkey's nose, before bringing the monkey into the laboratory. The distance between the camera and the nose of the monkey was then confirmed once the monkey was situated in the laboratory with the head fixed. The experiment began 10 min after attaching the electrodes, to allow the monkey to fully adapt to the situation (Nakayama et al., 2005; Kuraoka & Nakamura, 2011). Each session consisted of two phases lasting two minutes each: (1) the pre-stimulation period, i.e. rest condition, during which the monkey stayed quiet without any physical, auditory or visual contact with the experimenter. In the text, the pre-stimulation period is indicated as rest condition; and (2) the stimulation period, during which the experimenter applied the sweeping stimulation on the back of the monkey (approximately for 5 cm of the back). The stimulation period consisted of one of the three different motions: sweep at a speed of 1–3 cm/sec (slow speed); sweep at 5–10 cm/sec (medium speed); or sweep at 16–20 cm/sec (fast speed). Therefore, we conducted three different sessions: rest–sweep at a speed of 1–3 cm/sec, rest–sweep at a speed of 5–10 cm/sec, and rest–sweep at a speed of 16–20 cm/sec. The body part stimulated (the back) and the sweeping speeds were chosen based on the results of our previous study (Grandi, Roda & Ishida, 2015). Between each session, a period of 30 sec was included in order to minimize any potentially overlapping effect. The order of the sessions was randomly selected by the experimenter. The number of sessions for each day was chosen day-by-day, in accordance with the monkey's behavior. In total we conducted 12 trials for the sweep at 1–3 cm/s, and 9 trials each for the sweeps at 5–10 cm/sec and 16–20 cm/sec. After each day of recording, the monkey was returned to its own cage, where he fed and drank.

2.3 Data Collection

The ECG recordings were collected with surface electrodes (Medtronic®), while Spike2 was used to acquire the signal at the 1000 Hz sample rate. Each condition was recorded for 2 minutes (at least 90 seconds), with a 1 minute period analyzed. The RR interval values (time between two consecutive R-Waves detection; msec) were extracted with a custom script and exported as a text file to the Kubios HRV software (version 2.1; Biosignal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kupio, Finland) to obtain the HR (beats/min) and HRV parameters. For the frequency domain analysis of the HRV, the power spectrum was obtained with a fast Fourier transform-based method (FFT; Welch's periodogram: 256 points windows with 50% overlap). We considered the power of the high frequency (HF; 0.15–0.5 Hz) bands, expressed in absolute values (msec^2). The intervals of the HF bands were in accordance with the literature (Grandi & Ishida, 2015; Grandi, Roda & Ishida, 2015; Uchiyama, Othani & Ohta, 2007).

In the time domain of the HRV, we evaluated the square root of the mean of the squares of the successive differences (RMSSD; msec) between adjacent RRs, with the measurement of the short-term variation estimating the high frequency variations in HR, and therefore the activity of the parasympathetic nervous system (Heart Rate Variability Standards of Measurement, Physiological Interpretation, and Clinical Use, 1996; Malik, 1997).

For the thermal recording, we used a portable IRT camera (NEC Avio TVS500; Nippon Avionics Co., Ltd., Tokyo, Japan) with a standard optic system, a resolution of 320x240 pixels, an emissivity of 0.97, and an accuracy of 0.04° C. It was placed 80 cm in front of the monkey's face and the nose skin temperature was recorded approximately every 20 seconds. The images were analyzed with Grayess IRT Analyzer® PRO version 6.0 software, with each image corrected for environmental and reflected temperature. A total of 875 images were collected.

2.4 Statistical Analyses

For each condition (rest and the three sweep types), the mean value of the HR and each HRV parameters for the central 1 min of the recorded time was calculated. Concerning the IRT analysis, the optimum images within the recorded time were selected for each condition. Each image was considered by experimenter as optimum if perfectly focused, sharp and the nose must not be covered by hands. Then, the mean temperature value of the nose was calculated according to the respective condition.

For both the HR and HRV, and the thermal data, the Friedman test ($p < .05$) was performed in order to compare the three rest conditions, since the similarity between them was a prerequisite for evaluating the effect of stimulation. The rest conditions should be equal among them, since the monkey did not know nor expect which speed the experimenter would apply (see paragraph 3.1). The Wilcoxon test ($p < .05$) was performed for thermal and cardiac data in order to compare the three sweep conditions (at the three speeds), and the relative rest (pre-stimulation) condition. This analysis was performed to allow evaluation of the effect of sweeping at the different speeds in comparison to the rest condition, where no physical contact happened (see paragraph 3.1). The Kruskal-Wallis Test ($p < .05$) was then performed to compare the sweeping conditions at the three speeds each other. The aim of this analysis was to investigate whether sweeping at different speeds would modulate the nose temperature and/or the HR and/or HRV differently (see paragraph 3.2).

Regarding the thermal data, we additionally performed correlation analysis (Spearman's rank correlation), in order to ensure that the temperature of the nasal region was not dependent upon the ambient temperature in the room, for the rest and the three sweeping conditions (Nakayama et al., 2005). For the correlation analysis we compared the ambient temperature with the nose temperature of the monkey during the rest condition, the sweep at slow, medium and fast speed. In this analysis the rest condition was considered as the sum of the rest prior the sweep at slow, medium and fast speed (see paragraph 3.3). We considered the sum of the three rest conditions, since the rest conditions that preceded each sweeping did not differ each others (verified with Friedman Test, see above).

3. Results

3.1. The sweep determined a change in the heart rate, heart rate variability and nose skin temperature in comparison to the rest condition

A photograph of the monkey and two typical facial thermal images that were obtained during rest condition and during sweep with medium speed condition are shown in Fig. 1A, B and C, respectively. The temperature of the nose (marker "2" in the photo 1B and 1C) was lower during the sweep with medium speed (Fig. 1C) than during the rest condition (Fig. 1B).

The rest condition was the pre-stimulation period. Since during pre-stimulation period there was any physical contact between monkey and experimenter, we referred it as rest condition. Since during the rest conditions, that preceded each sweeping condition, the monkey did not expect any stimulation, nor if it did occur, did he know which of the three sweep types he would receive.

Consequently, we verified that the rest conditions that preceded each sweeping did not differ from each other in respect to the nasal temperature, HR and HRV (see paragraph 2.4; Friedman Test, $p < .05$). The comparison among the three rest conditions (rest prior the sweep slow, sweep medium and sweep fast) failed to underline any significant difference among them (medians rest prior the sweep slow: HR=95.18 beats/min; RMSSD=89.56 msec; HF=3156.9 $msec^2$. Medians rest prior the sweep medium: HR=94.86 beats/min; RMSSD=104.78 msec; HF=2051.15 $msec^2$. Medians rest prior the sweep fast: HR=89.92 beats/min; RMSSD=78.03 msec; HF=2626.51 $msec^2$. Friedman Test, HR: $\chi^2 = 0.29$; df =1; $p= .87$; RMSSD: $\chi^2 = 3.71$; df =1; $p= .16$; HF: $\chi^2 = 5.43$; df =1; $p= .07$; Temperature of the nose: $\chi^2 = 3.25$; df =1; $p= .19$).

Once we verified the absence of any differences among the three baselines, we then moved on to investigate the effect of each sweep in relation to the relative baseline (see paragraph 2.4; Wilcoxon test, $p < .05$) . The HR (Fig. 2A) was statistically higher during rest condition than during stimulation for each comparison: rest-slow speed (medians: rest=95.18 beats/min; slow speed=70.74 beats/min. Wilcoxon test, N=12; Z=3.06; $p= .02$); rest-medium speed (medians: rest=94.86 beats/min; medium speed=84.52 beats/min. Wilcoxon test, N=9; Z=2.67; $p= .007$); rest-fast speed (medians: rest=89.919 beats/min; fast speed: 74.69 beats/min. Wilcoxon test, N=9; Z=2.43; $p= .015$). The HF (Fig. 2A) was statistically higher during the rest condition than during the stimulation, for each comparison: rest-slow speed (medians: rest=3156.92 $msec^2$; slow speed=382.91 $msec^2$. Wilcoxon test, N=12; Z=2.75; $p= .006$); rest-medium speed (medians: rest=2051.15 $msec^2$; medium speed=3420.26 $msec^2$. Wilcoxon test, N=9; Z=2.07; $p= .038$); rest-fast speed (medians: rest=2626.51 $msec^2$; fast speed=289.87 $msec^2$. Wilcoxon test, N=9; Z=2.55; $p= .015$). The RMSSD (Fig. 2A) was statistically higher during the rest than during the stimulation, for comparisons rest-slow speed (medians: rest 89.56 msec; slow speed=45.77 msec. Wilcoxon test, N=12; Z=2.19; $p= .03$) and rest-fast speed (medians: rest=78.03 msec; fast speed=22.67 msec. Wilcoxon test, N=9; Z=2.67; $p= .008$). Nevertheless, we failed to identify any difference between the rest and sweeping at the medium speed in terms of RMSSD (Fig. 2A. Medians: rest=104.78

msec; medium speed=123.13 msec) speed. The nose temperature (Fig. 2B) was statistically lower during the rest than during the sweep for the comparisons: rest-slow speed (medians: rest=34.52°C; slow speed=35.97°C. Wilcoxon test, N=12; Z=2.27; *p*= *.02*); rest-medium speed (medians: rest=35.76°C; medium speed=36.7°C. Wilcoxon test, N=9; Z=2.67; *p*= *.008*), but not for the rest-fast sweep (medians: rest=35.26 °C; fast sweep=34.47°C).

3.2. The sweep performed with speed of 5-10 cm/sec determined increment of HRV and nose temperature in comparison to the sweep at lower and higher speed

The comparison among the three sweeping conditions (see paragraph 2.4; Kruskal-Wallis Test; *p*< *.05*) underscored that the HR did not differ among the three sweeps (medians slow speed= 70.74 beats/min; medium speed= 84.52 beats/min; fast speed= 74.69 beats/min). On the contrary, the RMSSD and the HF were statistically higher during the sweeping at medium speed than during the sweeping at both slow and fast speed (medians medium speed: RMSSD=123.13 msec; HF=3420.26 msec^2. Medians slow speed: RMSSD=45.77 msec; HF=382.91 msec^2. Medians fast speed: RMSSD=22.67 msec; HF=289.87 msec^2. Kruskal-Wallis Test medium speed vs slow speed; RMSSD: N=30; *p*= *.0009;* Z=2.69; and HF: N=30; *p*= *.003;* Z=2.75. Kruskal-Wallis Test medium speed vs slow speed, RMSSD: N=30;Z=2.69; *p*= *.02*; and HF: N=30; Z=2.75; *p*= *.02*. Kruskal-Wallis Test medium speed vs fast speed, RMSSD: N=30; Z=3.64; *p*< *.001*; and HF: N=30; Z=3.21; *p*= *.004*). Moreover, the last two sweeping conditions, sweep at fast and slow speed, did not differ each other (Fig. 3A).

The nose temperature (Fig. 3B) was statistically higher during sweeping at the medium speed than during the sweep at the fast speed (medians: medium speed= 36.70 °C; fast speed=34.47 °C. Kruskal-Wallis Test medium speed vs fast speed, N = 30; Z=2.88; *p* = *.016*), but there was no difference in the temperature during the sweep at the slow speed, while there was no difference between the temperatures at the slow and fast speeds.

3.3. The changes of the monkey's nose skin temperature depend on the condition

In order to verify that the observed changes of the nose temperature did not depend on the room temperature, instead on the condition, we performed a correlation analysis (see paragraph 2.4). The correlation analysis (Fig. 4) between each condition (the rest prior the sweep slow and medium and fast; sweep slow; sweep medium; sweep fast) and the room temperature during each of them underlined the absence of any relationship between the room temperature and that of the monkey's nose during each condition. In detail, there was no correlation between the room temperature and the monkey's nose temperature during rest condition (medians: rest=35.29°C; room temperature=26.60°C. Spearman's rank correlation coefficient, N=30; $r_s=0.179;\ p=.344$), during sweep with medium speed (medians: sweep medium speed=36.70°C; room temperature=27°C. Spearman's rank correlation coefficient, N=9; $r_s=0.456;\ p=.217$) and during sweep with fast speed (medians: sweep fast speed=34.47°C; room temperature=26.10°C. Spearman's rank correlation coefficient, N=9; $r_s=-0.452;\ p=.222$). During sweep slow the room temperature remained unvaried and the presence of a constant variable determined the impossibility to run the correlation. Nevertheless, since during the sweep slow the temperature of the room but not the temperature and of the monkey's nose did not change, it is possible to assume the absence of relationship between it and the monkey's nose skin temperature during the condition.

4. Discussion

The results presented here underlined that both the cardiac- and temperature-related physiological parameters were affected by the sensory tactile stimulation in non-human primates in the experimental situation. In particular, we tested the effects of sweeping the back of a rhesus monkey, performed by a familiar person (experimenter) with three ranges of speed, *i.e.* 1-3 cm/sec, 5-10 cm/sec and 16-20 cm/sec. When compared with the rest condition, the sweeping the back determined the decrement of HR and increment of HRV and of the nose skin temperature independently of the speed with which was applied. The comparison of the three sweeps underlined that the sweep performed with a speed of 5–10 cm/sec determined an increment of HRV and of the temperature around the nose of the monkey, in comparison to the sweeping performed with a speed of 1-3 cm/sec or 16-20 cm/sec. Importantly, concerning the temperature changes, the correlation analysis between room temperature and the nose skin temperature showed the absence of any relationship between the two measurements. Consequently, it is possible to assume that all the observed changes in the nose temperature were correlated to the relative condition, and not to the room temperature, that could affects the body temperature.

In a previous study (Grndi, Roda & Ishida, 2015), we demonstrated that, when compared with the rest condition, the sweeping back with speeds of 5 and 10 cm/sec determined a decrement of HR and increment of HRV. On the contrary the sweeping performed with speeds of 0.5-1 cm/sec and 20 cm/sec determined a decrement of HR and of HF, without reflecting any changes in the RMSSD. Moreover, we also showed that the sweeping performed with speed of 5 cm/sec and 10 cm/sec, determined an increment of HRV and a decrement of HR in comparison to the sweep performed with speed of 0.5-1 cm/sec and 20 cm/sec. The results present here can only partially be comparable with that study. Indeed, it is possible to compare the comparison between rest condition and sweeping performed with medium speed (5-10 cm/sec). The other two speeds were different and this render difficult the comparison between the two studies. Here we confirm the decrement of HR during the sweep performed with a speed of 5-10 cm/sec in comparison to the rest condition. Nevertheless, we failed to observe the increment of HF and RMSSD. This discrepancy could be determined by the condition *per se*, since in the present study we tested a range of speed, varied from 5 to 10 cm/sec, while in the previous study we tested more precisely the two speeds separately. Even we confirm the results of HR, the time domain (RMSSD) and the frequency domain (HF) parameters could be affected differently if considering the effect of a speed of 5 cm/sec or 10 cm/sec, or if considering the effect of a speed that can vary from 5 to 10 cm/sec and it is not fixed. Deeper investigation will be necessary in order to understand this discrepancy. Taken together the discrepancies and the confirmed results, we believe that the data of the present study reflect the positive cardio-physiological effect of the sweeping back with speed of 5-10 cm/sec, as the sweep back with speed of 5 cm/sec and 10 cm/sec.

Concerning the thermal results, it is reported the increment of the nose skin temperature of monkeys during positive situation (*e.g.* playing; Ioannous, Chotard & Davila-Ross, 2015) and the decrement, on the contrary, during negative situations (Stewart et al., 2007; Travain et al., 2015). These studies support the hypothesis that the sweeping performed at medium speed is a positive stimulation for the monkey, since determined an increment of the nose skin temperature in comparison to the rest condition and in comparison to the sweep performed with fast speed. Nevertheless, we observed an increment of the nose temperature also during the sweep performed with slow speed in comparison to the rest condition, parallel to the absence of difference between the sweep performed with slow and medium speed.

The IRT represents an emergent and important non-invasive contemporary technique to facilitate investigation of the temperature changes of different body parts in relation to differing emotions. Notwithstanding the many human (Ioannous, Gallese & Merla, 2014, Nummenmaa et al., 2014; Ioannou et al., 2013; Cacioppo, Gardner & Bernston, 1999; Merla & Romani, 2007) and animal (Stewart et al., 2007; Travain et al., 2015) studies, there is little evidence related to non-human primates. Moreover, there is no evidence concerning the effect of affiliative touch on the peripheral body temperature, either in human, in animals or in non-human primates. Concerning the use of IRT in non-human primates, despite being a non-invasive technique, there are only three studies in literature that analyzed body temperature in relation to emotional stimuli. These revealed that negative situations and emotions determined a decrement in the nose temperature (Nakayama et al., 2005; Kuraoka & Nakamura, 2011), while positive situations such as playing determined an increment in the nose temperature of the monkeys (Ioannous, Chotard & Davila-Ross, 2015). Even if in the present study the monkey had its head restrained, that represents an unnatural condition for the animal, the non-invasiveness of the IRT should be considered since it did not request any other constrain. Moreover, even in two (Nakayama et al., 2005; Kuraoka & Nakamura, 2011) of the three previously cited studies the monkeys had the head restrained, as in our one, while the third study (Ioannous, Chotard & Davila-Ross, 2015) underlined that it is possible to perform IRT recording without restraining the head of the monkeys. However, it guarantees to easily measure exactly the same skin point, with high precision, that would be more otherwise difficult if monkey was allowed to move the head.

Taken all the data together, the changes of the HR and HRV parameters across the three speeds did not exactly reflected the change of the thermal data. In absence of HR variation, HRV could be explained by the evidences that psychological states may have an impact on the sympathovagal balance, detectable through HRV analysis and in the absence of any palpable changes in the mean of HR (Tiller, McCraty & Atkinson, 1996). Nevertheless, there are not evidences related to the correlation between body temperature and HR and/or HRV during an emotional situation or during the interpersonal interaction between conspecifics. Therefore, we believe that deeper investigation will be necessary to understand the correlation between physiological parameters during the rest condition, and the effect of positive and negative interpersonal interactions, both in human and non-human primates.

Studies on humans demonstrated that the caress to the hairy side of the skin at a speed of CT-fiber modulation determined positive vagal modulation, regarding increments of the HR and HRV of the recipient person. Because of these positive physiological effects, this kind of tactile stimulation is utilized in clinical therapy to reduce stress and elevated the welfare of both healthy people (5Lindgren et al., 2010) and patients with (for example) depression or chronic pain (Diego & Field, 2009; Field, 2014; Tsao, 2007; Billhult et al., 2009; Belinda et al., 2008; Schroeder, Doig & Premkumar, 2014; Russell et al., 2008). Moreover, pleasant touch determines positive physiological effects in preterm infants. In fact, it was reported that the massage of preterm neonates at 31 weeks led to increased weight gain, reduced irritability and sleep disturbance, and a decrement of the HR and increment of vagal activity (Field, 1986). Behavior analysis revealed that this touch is perceived as pleasant and neuroimaging studies reported that it is coded as affiliative at the central nervous system level (Olausson et al., 2002; Olausson et al., 2008; McGlone et al., 2007). Our results demonstrated that the HR and HRV are in line with the literature on human studies, since the sweeping at 5–10 cm/sec determined the decrement of HR and the increment of HRV, and we could suppose that it is perceived by the monkey as a positive stimulation. Therefore, such stimulation could be useful in order to improve the welfare of laboratory animals and to reduce the stress they could be exposed to. Indeed, in our previous studies, we proposed this stimulation (Grandi, Roda & Ishida, 2015) and the picking-grooming like (Grandi & Ishida, 2015) to improve the welfare of experimental monkeys, based on physiological results. Here we stronger support this suggestion.

The novelty of the present data is represented by the evidence of the increment of nose temperature during the medium speed sweep, that together with the evidence of the increment of HRV led to hypothesize that the medium speed sweep could be considered as a positive tactile stimulation for the monkey and could improve the welfare of experimental monkeys. In fact, it is extremely important to maintain and guarantee not just the good conditions in terms of physical health of experimental animals, by means of veterinarian visits, but also the welfare in terms of low levels of stress. Indeed, the experimental animals under stress cannot work during experimental sessions. Finally, a good interaction with experimenters is essential for any research using animals. Recently Viktor and Annie Reinhardt (Reinhardt & Reinhardt, 2008) have hypothesized that the positive physical contact with personnel could be a method to increase the welfare of singly housed non-human primates. The sweeping and the picking-grooming like could be used for this purpose. Interestingly, the two above mentioned stimulations constitute the two hand motion of the social allo-grooming, the social behavior among non-human primates, with demonstrated affiliative meaning and positive autonomic effects (Boccia, 1989; Aureli, Preston & de Waal, 1999; Gust et al., 1993; Shutt et al., 2007; Dunbar, 2010).

In conclusion, the data presented here represent the first evidence of the changes in peripheral body temperature during affiliative touch at the speed of 5–10 cm/sec in an experimental male rhesus monkey, and the correlation among body temperature, HR, and HRV. We believe that the present study represents an important starting point in order 1) to analyze the effect of affiliative touch through activating the CT-fibers in terms of body temperature in humans and non-human primates; 2) to further investigate the correlation among body temperature, HR, and HRV; 3) to deeply investigate the homology between humans and non-human primates' affective system that is mediated by CT fibers; 4) to improve the use of non-invasive methods to monitor the well-being of experimental non-human primates, such as IRT, HR, and HRV; and 5) to improve the welfare of experimental animals sweeping the back at the speed of 1–10 cm/sec.

Finally, even if the present study represents a case report relating the autonomic changes that manifested during a tactile stimulation in non-human primates, we believe it could be an important starting point in order to more closely examine the effect of affiliative touch on the body temperature in both human (the caress) and non-human (grooming) primates.

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7. Figures legends

Figure 1. An example of changes in the nasal skin temperature of the monkey. **A)** A photograph of the monkey. **B)** and **C)** Thermal images of Rhesus monkey, with remark points. The nasal skin temperature in **B),** rest condition, is higher than in **C),** medium speed sweeping. The marker "2" indicates the point considered for the value of the nose temperature.

Figure 2. A) The graphs represent the HR, the time domain parameter RMSSD and the frequency domain parameter HF for each pair rest-slow, rest-medium and rest-fast. **B)** The graphs represent the nasal skin temperature (°C), for each pair rest-slow, rest-medium and rest-fast. In **A)** and **B)** the big black squares represent the mean of each condition ± the standard error, the small grey squares inside represent the mean of each condition, the vertical bars indicate the mean ± the standard deviation. The asterisks represent the statistically significant difference between the conditions ($p < .05$; Wilcoxon Test, for each parameter and each comparison).

Figure 3. A) The graphs represent the HR, the time domain parameter RMSSD and the frequency domain parameter HF for the comparison among the 3 speeds: slow, medium and fast. **B)** The graphs represent nasal skin temperature (°C), for the comparison among the 3 speeds: slow, medium and fast. In **A)** and **B), t**he big black squares represent the mean of each condition ± the standard error, the small grey squares inside represent the mean of each condition, the vertical bars indicate the mean ± the standard deviation. The asterisks represent the statistically significant difference between the conditions ($p < .05$; Kruskal-Wallis Test, for each parameter).

Figure 4. Correlation map among the room temperature (Room T) during each condition and the monkey's nose temperature in each conditions (Spearman's rank correlation). Room T during rest indicates the room temperature during the rest condition (sum of the rest condition of the three sweeping), Room T during sweep slow/medium/fast indicates the room temperature during the sweep slow/medium/fast; Nose T during rest indicates the monkey's nose temperature during the rest condition (sum of the rest condition of the three sweeping), Nose T during sweep slow/medium/fast indicates the monkey's nose temperature during the sweep slow/medium/fast. **Corr.Sign. $p< .01$ (2-tails). *Corr.Sign. $p< .05$ (2-tails). .[a] the room temperature variable is constant, the analysis is not possible.

Figure 1(on next page)

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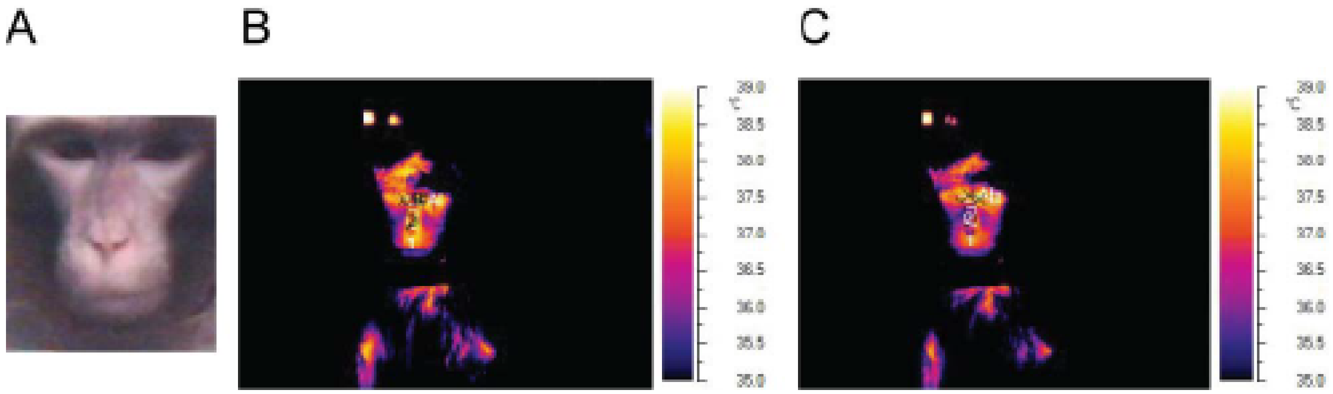
A
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C
°C
39.0
38.5
38.0
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36.0
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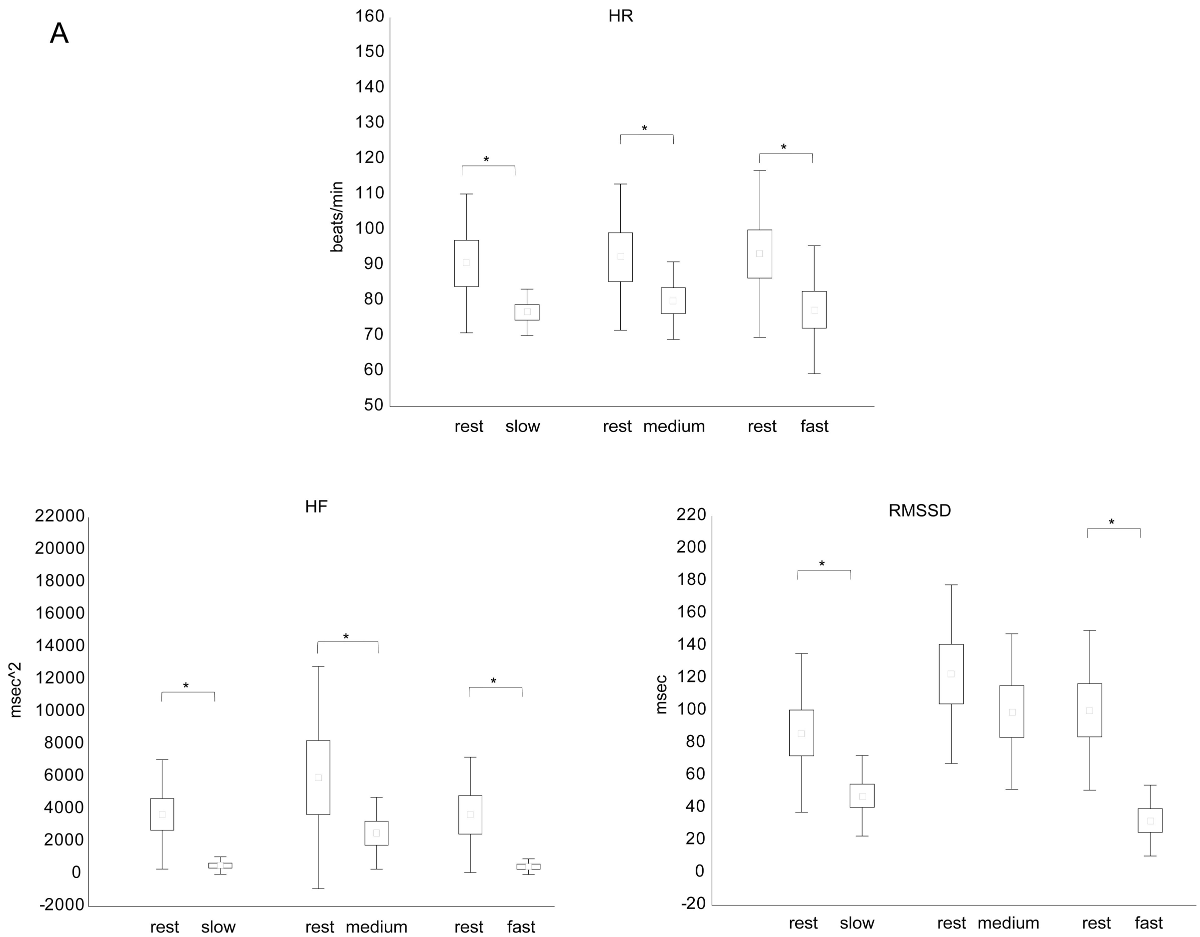
Figure 2(on next page)

Figure 2.

A) The graphs represent the HR, the time domain parameter RMSSD and the frequency domain parameter HF for each pair rest-slow, rest-medium and rest-fast. **B)** The graphs represent the nasal skin temperature (°C), for each pair rest-slow, rest-medium and rest-fast. In **A)** and **B)** the big black squares represent the mean of each condition ± the standard error, the small grey squares inside represent the mean of each condition, the vertical bars indicate the mean ± the standard deviation. The asterisks represent the statistically significant difference between the conditions ($p < .05$; Wilcoxon Test, for each parameter and each comparison).

A



B

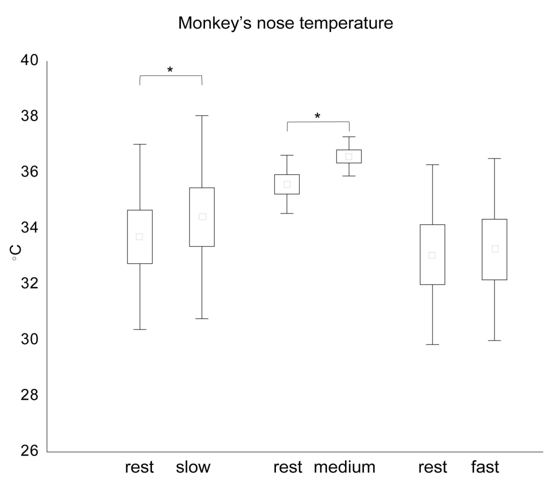


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Figure 3(on next page)

Figure 3.

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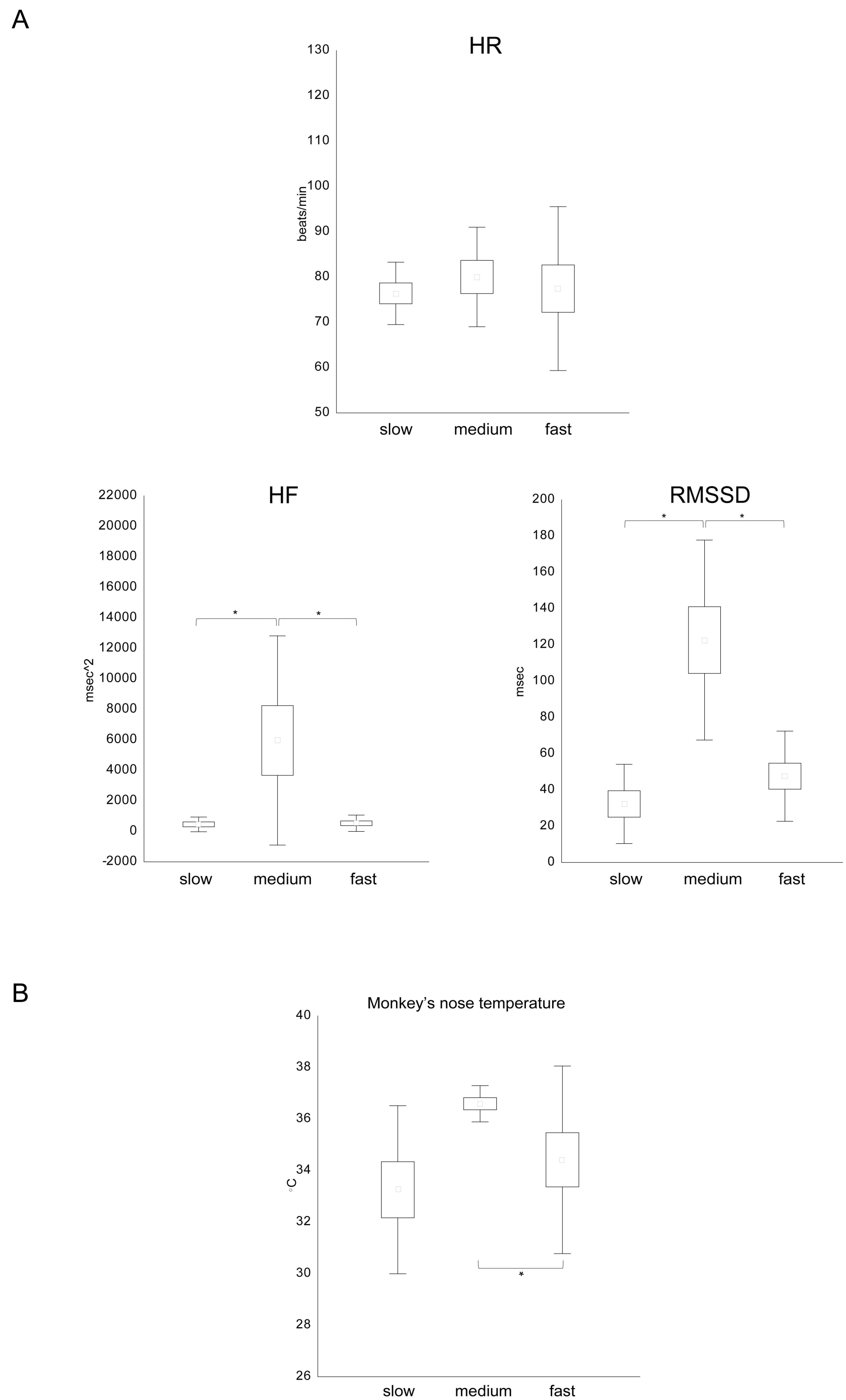
A
HR
beats/min
130
120
110
100
90
80
70
60
50
slow
medium
fast
HF
msec^2
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
*
*
slow
medium
fast
RMSSD
msec
200
180
160
140
120
100
80
60
40
20
0
*
*
slow
medium
fast
B
Monkey's nose temperature
°C
40
38
36
34
32
30
28
26
*
slow
medium
fast

Figure 4(on next page)

Figure 4.

Correlation map among the room temperature (Room T) during each condition and the monkey's nose temperature in each conditions (Spearman's rank correlation). Room T during rest indicates the room temperature during the rest condition (sum of the rest condition of the three sweeping), Room T during sweep slow/medium/fast indicates the room temperature during the sweep slow/medium/fast; Nose T during rest indicates the monkey's nose temperature during the rest condition (sum of the rest condition of the three sweeping), Nose T during sweep slow/medium/fast indicates the monkey's nose temperature during the sweep slow/medium/fast. **Corr.Sign. *p< .01* (2-tails). *Corr.Sign. *p< .05* (2-tails). .[a] the room temperature variable is constant, the analysis is not possible.

		Nose T during rest	Nose T during sweep slow	Nose T during sweep medium	Nose T during sweep fast
Room T during rest	Coef.Corr Sign. (2-tails) N	0.179 0.344 30	-0.024 0.94 12	0.52 0.152 9	0.087 0.825 9
Room T during sweep slow	Coef.Corr Sign. (2-tails) N	.a .a 12	.a .a 12	.a .a 9	.a .a 9
Room T during sweep medium	Coef.Corr Sign. (2-tails) N	0.183 0.638 9	-0.091 0.815 9	0.456 0.217 9	0.126 0.766 8
Room T during sweep fast	Coef.Corr Sign. (2-tails) N	-0.323 0.397 9	-0.581 0.101 9	0.481 0.227 8	-0.452 0.222 9

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