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Tocco piacevole nei primati non umani: dati preliminari sull'effetto sul sistema nervoso autonomico e codifica a livello del sistema nervoso centrale

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Pleasant sweeping grooming touch in Rhesus monkey: effects on autonomic nervous system and its codification in posterior insular and secondary somatosensory cortex

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A Voi, Sospiri di Luce e Lacrime di Buio
A Voi che Avete Distrutto
A Voi che Avete Costruito
A Voi a cui la Luna deve la sua Vita
A Voi a cui la Luna deve la sua Rinascita
A Voi a cui la Luna deve la sua Bellezza

Alle Eterne Stelle
Alla Nuova Luna

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Abstract

The sense of touch assumes a critical importance in daily life, since allow us to discriminate and to haptically explore and identify objects, integrating or not with other sensory information. This is the *sensory-discriminative* aspect of the touch.

Nevertheless we also used the sense of touch during many social interactions, both positive, *e.g.* to caress a familiar person, and negative, *e.g.* to move away a stranger person. This aspect of the touch is *the motivational-affective* dimension, that determines the codification of the emotional valence and the meaning of the tactile interaction. This component allow us to create and maintain social bonds in relation to the meaning that the touch assumes. If a familiar person caress us we perceive this touch as pleasant and encode it as affiliative. This positive affective touch is commonly define as *Social Touch*.

The discriminative aspects of touch have been well-studied, since historically, the role of the touch has been consider discriminative, while the affective aspects have only recently been investigated, even its importance in the social interaction.

The static touch responsible of the discriminative aspect activates the large myelinated low threshold mechanoreceptors (LTMRs) and modulates both primary and secondary sensory cortices. This allows the codification at level of the central nervous system of the touched objects characteristics.

Instead, the affiliative touch in humans is a dynamic touch occurred on the hairy side of the skin with a velocity of 1-10 cm/sec and activates the C unmyelinated low threshold mechanoreceptors (CT fibers or C-LTMRs). This dynamic tactile stimulation determines positive physiological effects, *e.g.* decrement of heart rate (HR) and increment of heart rate variability (HRV), and the modulation of brain regions involved in the coding of the affiliative valence of the sensory peripheral stimulation. In particular, the posterior insular cortex has a central role in processing gentle touch following activation of CT fibers.

The sense of touch with its two dimensions is present not just among humans, but also among non-human primates. In fact, all non-human primates used the discriminative aspect of the touch to identify objects and food and the emotional aspect during the social interactions, both negative as fight and positive, as grooming.

The mechanisms of the codification of the discriminative component in non-human primates are similar to the humans ones. Nevertheless, the state of art related the mechanisms behind the codification of pleasant touch in non-human primates is very poor. It is well known that the C-LTMRs are present also in the hairy side of the skin of non-human primates, but nowadays there are any studies related the correlation between the C-LTMRs and the pleasantness, as in humans. Recently it was proposed (Dunbar, 2010) the role of C-LTMRs during the sweeping occurred during

allogrooming. Like gentle caress for humans, in the same way allogrooming, is a social affiliative behavior for non-human primates. The sweeping is the hand action that the agent monkey perform during grooming to move the fur of the receipt monkey in order to see the site of skin to be picked and remove ectoparasites and vegetation trapped in the fur. The sweeping is therefore similar to the human caress, but performed with the unique aim to move the fur and clean it. Instead, the human caress is an affiliative gesture. According to the hypothesis of the C-LTMRs' role in the sweeping, this action should have not just hygienic function but also an affiliative meaning, as caress in human.

Nevertheless, up to now there is no direct evidence in support of this hypothesis, therefore 1) is the velocity of the sweeping among monkeys inside the optimal range of 1-10 cm/sec that activate the CT fibers, as affiliative caress in humans?; 2) are the effects of autonomous nervous system of the sweep similar to those of human caress?; 3) is insular cortex modulated and involved in the codification of the affiliative aspect of the sweep, as during pleasant touch in humans?

The aim of the present work was to answer to those questions, investigating different aspects of the sweeping in non-human primates, in particular in Rhesus monkeys. The answers could allow to speculate the similarity of the monkeys pleasant sweeping and the humans gentle caress.

In particular, we investigated the following aspects. In the *Study 1* we evaluated the velocity of real sweeping with a preliminary kinematic analysis from videotapes recording in a group of free ranging Rhesus monkeys. In the *Studies 2* and *3*, we investigated the autonomic effects of the sweep while in the *Study 4* the codification of sweeping at central nervous system level. In the *Studies 2*, *3* and *4* the sweep was performed by experimenter with different speeds to a male Rhesus monkey in a typical experimental situation, *i.e.* in the laboratory, seated in the primate chair with the head fixed. The speeds were chosen in relation to the optimal and non-optimal velocities of the activation of CT fibers in humans and taking into account also the results of *Study 1* therefore the velocity of real sweep that should be pleasant for the monkey. In particular, we tested the autonomic responses in term of HR and HRV, as index of vagal modulation (*Study 2*), and in term of nose skin temperature (*Study 3*). Finally, in the *Study 4* we studied the role of the secondary somatosensory cortex and of the disgranular and granular insular cortex in the codification of the sweeping.

The preliminary data here presented show that 1) (*Study 1*) the velocity of sweeping grooming touch among free ranging monkeys is in the range of the optimal velocity (1-10 cm/sec) to activate the CT fibers in humans (9.31 cm/sec); 2) (*Study 2*) a sweep on the back of a male Rhesus monkey performed with velocities of 5 cm/sec and 10 cm/sec determined a decrement of the HR and increment of the HRV; 3) (*Study 3*) the sweep on the back of a male Rhesus monkey occurred with a velocity in the range of 5-10 cm/sec determined the increment of the nose skin temperature, and 4)

(*Study 4*) the sweep performed with a velocity in range 5-13 cm/sec, but not 1-5 cm/sec, determined the modulation of both insular and secondary somatosensory cortices.

These results support the Dunbar's hypothesis above mentioned related the involvement of CT fibers during sweeping grooming. In fact, this motion is performed at similar velocity of pleasant human touch that activates CT fibers (1-10 cm/sec), determines the same positive physiological effects in terms of HR (decrement) and HRV (increment) and the modulation of same brain regions (the insular cortex). Furthermore for the first times we investigated the effect of pleasant touch on the body temperatures, underlined that the pleasant sweep determines the increment of the monkey's nose skin temperature.

The present study represents the first indirect evidence of the hypothesis related the modulation of CT fibers system during pleasant sweeps, and of the representation of the affiliative gentle sweeping at both autonomic and central nervous system in non-human primates. These preliminary data highlight the similarity between human and non-human primates social touch system.

Nevertheless, we found some discrepancy with human studies. In fact, the mean velocity of the real sweeping is of 9.31 cm/sec, almost the upper limit (10 cm/sec) of range of speed that activates human CT fibers. Moreover, the positive autonomic effects, in terms of HR, HRV and nose skin temperature was obtained during the human sweep performed with velocities of 5 and 10 cm/sec, therefore at the upper limit of the optimal range that activates the human CT fibers. On the contrary, the sweep performed with velocities lower than 5 and higher than 10 cm/sec determined negative physiological effect. At central nervous system level, the insular cortex was selectively modulated during the sweep performed with velocity in the range 5-13 cm/sec. Human studies demonstrated that the optimal velocity to activate CT fibers, to determine the positive autonomic effects and to modulate the insular cortex is 1-10 cm/sec. Our results underscore instead that the optimal velocity seems to be 5-13 cm/sec.

Taken together the results and the highlight discrepancies, underscore the homology between human and human CT system, and the necessity of further studies in order to deeply investigate the real sweep among monkeys, to more precisely determine the optimal velocity of the C-LTMR modulation and to directly demonstrate their activation during sweeping by means of direct measurement of their activity. Studies in that direction will confirm the homology between human and non-human primate affective systems mediated by the CT fibers.

Finally, the present study could be an important starting point to explore the evolutionary mechanism behind the transformation of the sweeping among non-human primates, utilitarian action performed during grooming to clean others, to the caress, affiliative gesture among humans.

Riassunto

Il tatto assume un'importanza fondamentale nella vita quotidiana, in quanto ci permette di discriminare le caratteristiche fisiche di un oggetto specifico, di identificarlo e di eventualmente integrare le suddette informazioni tattili con informazioni provenienti da altri canali sensoriali. Questa è la componente sensoriale-discriminativa del tatto.

Tuttavia quotidianamente il tatto assume un ruolo fondamentale durante le diverse interazioni sociali, positive, come quando abbracciamo o accarezziamo una persona con cui abbiamo un rapporto affettivo e negative, per esempio quando allontaniamo una persona estranea dal nostro spazio peri-personale.

Questa componente è la cosiddetta dimensione affettiva-motivazionale, la quale determina la codifica della valenza emotiva che l'interazione assume. Questa componente ci permette di creare, mantenere o distruggere i legami sociali in relazione al significato che il tocco assume durante l'interazione. Se per esempio riceviamo una carezza da un familiare, questa verrà percepita come piacevole e assumerà un significato affiliativo. Questo tipo di tocco è comunente definito come Tocco Sociale (*Social Touch*).

Gli aspetti discriminativi del tatto sono stati ben caratterizzati, in quanto storicamente, il ruolo del tatto è stato considerato quello di discriminare le caratteristiche di ciò che viene toccato, mentre gli aspetti affettivi sono stati solo recentemente indagati considerando la loro importanza nelle interazioni sociali.

Il tocco statico responsabile dell'aspetto discriminante attiva a livello della pelle le grandi fibre mieliniche (Aβ), modulando a livello del sistema nervoso centrale le cortecce sensoriali, sia primarie che secondarie. Questo permette la codifica a livello del sistema nervoso centrale delle caratteristiche fisiche oggettive degli oggetti toccati.

Studi riguardanti le caratteristiche del tocco affiliativo sociale hanno messo in evidenza che suddetta stimolazione tattile 1) è un particolare tocco dinamico che avviene sul lato peloso delle pelle con una velocità di 1-10 cm/sec; 2) attiva le fibre amieliniche (fibre CT o C-LTMRs); 3) induce positivi effetti autonomici, ad esempio la diminuzione della frequenza cardiaca e l'aumento della variabilità della frequenza cardiaca; e 4) determina la modulazione di regioni cerebrali coinvolte nella codifica del significato affiliativo dello stimolo sensoriale periferico, in particolare la corteccia insulare.

Il senso del tatto, con le sue due dimensioni discriminativa e affiliativa, è quotidianamente usato non solo negli esseri umani, ma anche tra i primati non umani. Infatti, tutti i primati non umani utilizzano la componente discriminativa del tatto per identificare gli oggetti e il cibo e l'aspetto emotivo durante le interazioni sociali, sia negative come durante un combattimento, che positive, come durante i comportamenti affiliativi tra cui il *grooming*.

I meccanismi di codifica della componente discriminativa dei primati non umani sono simili a quelli umani. Tuttavia, si conosce ben poco dei meccanismi alla base della codifica del tocco piacevole affiliativo. Pur essendo ben noto che i meccanorecettori amilienici C-LTMRs sono presenti anche sul lato peloso della pelle dei primati non umani, attualmente non ci sono studi riguardanti la correlazione tra il tocco piacevole e la loro modulazione, come invece è stato ampiamente dimostrato nell'uomo.

Recentemente è stato ipotizzato (Dunbar, 2010) il ruolo delle fibre C-LTMRs durante il *grooming*, in particolare durante il cosiddetto swepping. Il *grooming* è costituito da due azioni motorie, lo *sweeping* e il *picking* che vengono eseguite in modo ritmico. Durante lo *sweeping* la scimmia agente muove il pelo della scimmia ricevente con un movimento a mano aperta, per poter vedere il preciso punto della pelle dove eseguire il *picking*, ovvero dove prendere la pelle a livello della radice del pelo con le unghie dell'indice e del pollice e tirare per rimuovere parassiti o uova di parassiti e ciò che è rimasto incastrato nel pelo. Oltre il noto ruolo igenico, il *grooming* sembra avere anche una importante funzione sociale affiliativa. Come la carezza nella società umana, cosi il *grooming* tra i primati non umani è considerato un comportamento. Secondo l'ipotesi di Dunbar l'attivazione delle C-LTMRs avverrebbe durante lo *sweeping* e questo porta a supporre che lo *sweeping*, come la carezza umana, costituisca una componente affiliativa del *grooming*, determinando quindi a contribuire alla sua codifica come comportamento sociale.

Fino ad ora non vi è però alcuna prova diretta a sostegno di questa ipotesi. In particolare, 1) la velocità cui viene eseguito lo *sweeping* è compatibile con la velocità di attivazione delle fibre CT nell'uomo e quindi con la velocità tipica della carezza piacevole di carattere sociale affiliativo (1-10 cm/sec)?; 2) lo *sweeping* induce la stessa modulazione del sistema nervoso autonomo in direzione della modulazione del sistema vagale, come il tocco piacevole nell'uomo, attraverso l'attivazione delle fibre CT?; 3) lo *sweeping* modula la corteccia insulare, cosi come il tocco piacevole viene codificato come affiliativo nell'uomo mediante le proiezioni delle fibre CT a livello dell'insula posteriore?

Lo scopo del presente lavoro è quella di testare l'ipotesi di Dunbar sopra citata, cercando quindi di rispondere alle suddette domande. Le risposte potrebbero consentire di ipotizzare la somiglianza tra lo *sweeping*, caratteristico del comportamento affiliativo di *grooming* tra i primati non umani e la carezza.

In particolare, abbiamo eseguito 4 studi pilota. Nello *Studio 1* abbiamo valutato la velocità con cui viene eseguito lo *sweeping* tra scimmie Rhesus, mediante una analisi cinematica di video registrati tra un gruppo di scimmie Rhesus. Negli *Studi 2* e *3* abbiamo valutato gli effetti sul sistema nervoso autonomo dello *sweeping* eseguito dallo sperimentatore su una scimmia Rhesus di sesso

maschile in una tipica situazione sperimentale. La stimolazione tattile è stata eseguita a diverse velocità, in accordo con i risultati dello *Studio 1* e degli studi umani che hanno dimostrato la velocità ottimale e non ottimale per l'attivazione delle C-LTMRs. In particolare, nello *Studio 2* abbiamo misurato la frequenza cardiaca e la variabilità di questa, come indice della modulatione vagale, mentre nello *Studio 3* abbiamo valutato gli effetti dello *sweeping* sul sistema nervoso autonomo in termini di variazioni di temperatura del corpo, nello specifico a livello del muso della scimmia. Infine, nello *Studio 4* abbiamo studiato il ruolo della corteccia somatosensoriale secondaria e insulare nella codifica dello *sweeping*. A questo scopo abbiamo eseguito registrazioni di singoli neuroni mentre la medesima scimmia soggetto sperimentale dello *Studio 2* e *3*, riceveva lo *sweeping* a due velocità, una ottimale per l'attivazione delle C-LTMRs secondo gli studi umani e i risultati dei tre studi sopra citati, ed una non ottimale.

I dati preliminari ottenuti, dimostrano che 1) (*Studio 1*) lo *sweeping* tra scimmie Rhesus viene eseguito con una velocità media di 9.31 cm/sec, all'interno dell'intervallo di attivazione delle fibre CT nell'uomo; 2) (*Studio 2*) lo *sweeping* eseguito dallo sperimentatore sulla schiena di una scimmia Rhesus di sesso maschile in una situazione sperimentale determina una diminuzione della frequenza cardiaca e l'aumento della variabilità della frequenza cardiaca se eseguito alla velocità di 5 e 10 cm/sec. Al contrario, lo *sweeping* eseguito ad una velocità minore di 1 cm/sec o maggiore di 10 cm/sec, determina l'aumento della frequenza cardiaca e la diminuzione della variabilità di questa, quindi il decremento dell'attivazione del sistema nervoso parasimpatico; 3) (*Studio 3*) lo *sweeping* eseguito dallo sperimentatore sulla schiena di una scimmia Rhesus di sesso maschile in una situazione sperimentale determina l'aumento della temperatura corporea a livello del muso della scimmia se eseguito alla velocità di 5-10 cm/sec. Al contrario, lo *sweeping* eseguito ad una velocità minore di 5 cm/sec o maggiore di 10 cm/sec, determina la diminuzione della temperatura del muso; 4) (*Studio 4*) la corteccia somatosensoriale secondaria e la corteccia insulare posteriore presentano neuroni selettivamente modulati durante lo *sweeping* eseguito ad una velocità di 5-13 cm/sec ma non neuroni selettivi per la codifica della velocità dello *sweeping* minore di 5 cm/sec.

Questi risultati supportano l'ipotesi di Dunbar relativa al coinvolgimento delle fibre CT durante lo *sweeping*. Infatti i dati mettono in luce che lo *sweeping* viene eseguito con una velocità (9.31 cm/sec), simile a quella di attivazione delle fibre CT nell'uomo (1-10 cm/sec), determina gli stessi effetti fisiologici positivi in termini di frequenza cardiaca (diminuzione) e variabilità della frequenza cardiaca (incremento) e la modulazione delle medesime aree a livello del sistema nervoso centrale (in particolare la corteccia insulare). Inoltre, abbiamo dimostrato per la prima volta che suddetta stimolazione tattile determina l'aumento della temperatura del muso della scimmia. Il presente studio rappresenta la prima prova indiretta dell'ipotesi relativa alla modulazione del

sistema delle fibre C-LTMRs durante lo *sweeping* e quindi della codifica della stimolazione tattile piacevole affiliativa a livello del sistema nervoso centrale ed autonomo, nei primati non umani. I dati preliminari qui presentati evidenziano la somiglianza tra il sistema delle fibre CT dell'uomo e del sistema C-LTMRs nei primati non umano, riguardanti il *Social Touch*.

Nonostante ciò abbiamo riscontrato alcune discrepanze tra i risultati da noi ottenuti e quelli invece ottenuti dagli studi umani.

La velocità media dello *sweeping* è di 9.31 cm / sec, rasente il limite superiore dell'intervallo di velocità che attiva le fibre CT nell'uomo. Inoltre, gli effetti autonomici positivi, in termini di battito cardiaco, variabilità della frequenza cardiaca e temperatura a livello del muso, sono stati evidenziati durante lo *sweeping* eseguito con una velocità di 5 e 10 cm/sec, quindi al limite superiore dell'intervallo ottimale che attiva le fibre CT nell'uomo. Al contrario, lo *sweeping* eseguito con una velocità inferiore a 5 cm/sec e superiore a 10 cm/sec determina effetti fisiologici negativo. Infine, la corteccia insula sembra essere selettivamente modulata dallo stimolazione eseguita alla velocità di 5-13 cm/sec, ma non 1-5 cm/sec.

Quindi, gli studi sul sistema delle fibre CT nell'uomo hanno dimostrato che la velocità ottimale è 1-10 cm/sec, mentre dai nostri risultati la velocità ottimale sembra essere 5-13 cm / sec.

Quindi, nonostante l'omologia tra il sistema delle fibre CT nell'umano deputato alla codifica del tocco piacevole affiliativo ed il sistema delle fibre C-LTMRs nei primati non umani, ulteriori studi saranno necessari per definire con maggiore precisione la velocità ottimale di attivazione delle fibre C-LTMR e per dimostrare direttamente la loro attivazione durante lo *sweeping*, mediante la misurazione diretta della loro modulazione. Studi in questa direzione potranno confermare l'omologia tra lo *sweeping* in qualità di tocco affiliativo piacevole tra i primati non umani e la carezza tra gli uomini.

Infine, il presente studio potrebbe essere un importante punto di partenza per esplorare il meccanismo evolutivo dietro la trasformazione dello *sweeping* tra primati non umani, azione utilitaria eseguita durante il *grooming*, a carezza, gesto puramente affiliativo tra gli uomini.

Introduction

The sense of touch

The sense of touch is a critical and necessary sense in daily life to our experience of the world, to haptically explore, identify and discriminate the features and the localization of a specific touched object, with eventually the integration with other sensory information. This is the *sensory-discriminative* aspect of the touch.

However the touch is also daily used during positive and negative social interactions, among individuals. We used to define this kind of touch inter-individual touch and it is frequently used to communicate both negative and positive messages, like aversion or comfort and support, respectively (Hertenstein *et al.*, 2006a and 2006b). The involved component in this case is the *motivational-affective* dimension, that determines the codification of the emotional valence and the meaning of the tactile interaction. This component allow us to create, maintain, modify or broke the social bonds in relation to the meaning that the touch assumes, *e.g.* aversive or pleasant. For example, if we perceived as aversive the touch received from a person, we are led to interrupt any relationship with him/her. On the contrary, a positive pleasant touch led us to create or reinforce the social bonds. Indeed, if the touch is perceived as pleasant it is commonly define as *Social Touch*, since a positive contact between conspecific determines the creation of social relationships.

Therefore, it is possible to identify two dimensions of the touch: the *sensory-discriminative*, that determines the codification of the characteristics of the objects and the *motivational-affective* dimension, that determines the valence of the touch.

The discriminative aspects have been well-studied, since historically, the role of the touch has been consider discriminative (Mountcastle, 2005), while the emotional aspect of skin-to-skin interpersonal touch has been given less attention and have only recently been investigated, even its importance in social interaction (Morrison *et al.*, 2010).

Generally the social related tactile stimulation, without any discriminative aspects, can be divided in four categories (Morrison *et al.*, 2010):

1. ***Simple touch***; it involves intentional brief tactile contact to a restricted localization on the body surface. It was demonstrated that a hand-to-hand touch increased a positive evaluation of other person, but also that the recipients of such simple touch are more compliant and unselfish. From these evidences it was hypothesized that such simple touch could be important in order to create future good cooperation among individuals (Fisher *et al.*, 1976; Hornik, 1992).

2. ***Protracted touch***; it involves longer skin-to-skin contact between individuals. It was demonstrated that holding the hand of other person can reduce the anxiety level (Coan *et al.*, 2006).

3. ***Dynamic touch***; it involves repetitive motion over the others skin from one to another point. This type of tactile stimulation is commonly known as moderate massage and caress and is used as a therapy to improve the wellbeing of patients with acute and/or chronic depression, chronic pain, cancer patients under chemo- and/or radiotherapy, to improve the immune system of premature infants (Lindgren *et al.*, 2010; Diego and Field, 2009; Tsao, 2007; Billhult *et al.*, 2009; Belinda *et al.*, 2008; Schroeder *et al.*, 2014; Russell *et al.*, 2008). Interestingly, it is instinctively performed with a specific pressure and velocity.

4. ***Tickling***; it represents a special category associates with playful but not daily behavioral context.

The discriminative and the social touch involves different mechanoreceptors that innervate the human skin (Table 1), each with specific physiological properties.

The main type of primary sensory neurons that mediate discriminative touch are the myelinated low threshold mechanoreceptive (LTMR) Aβ fibers, present on both the hairy and glabrous side of the skin. These fibers project via the dorsal column of the spinal cord (lamina III and V) to the ventral posterior lateral nucleus of the thalamus and from there to the first and secondary somatosensory cortices (SI and SII, respectively).

Glabrous skin	***Hairy skin***	***Adaptation***
Merkel SAI	Merkel SAI	Slow
Ruffini SAII	Ruffini SA II	Slow
Pacini FA II (PC)	Pacini FA II (PC)	Fast
Meissner FAI (RA, QA)	Hair follicle unit	Fast
	Field	Fast
	C-tactile CT (CLTM)	Intermediate

Table 1. *Mechanoreceptive afferents in human nerves from glabrous and hairy skin. Most acronyms refer to adaptation properties (fast, rapid, quick or slow adaptation). Acronyms within parenthesis have mainly been used in studies of non-human species. All afferents listed are myelinated and fast conducting (Aβ fibers) except CT units which are unmyelinated (C fibers). The table does not include nociceptive afferents that may respond weakly to innocuous skin deformation. The table entries refer to glabrous skin of the hand and hairy skin of the forearm and are based on recent investigations. However, there are microneurography studies suggesting a somewhat different innervation pattern in particular skin regions, the existence of subgroups, and one or two additional mechanoreceptor type (From Olausson et al., 2010).*

The pleasant touch determines the activation of low-threshold, slowly-conducting unmyelinated C-tactile (CT) afferent fibers ('C tactile' or 'tactile C') present on the hairy side of the skin. Once activated, the CT fibers project via lamina I/II of the spinothalamic tract to posterior/basal ventral medial nucleus, to posterior insular and orbitofrontal cortices (Olausson *et al.*, 2010), specific brain regions assigned to the codification of the emotional aspect of peripheral sensory stimulation.

Therefore, the Aβ- related and CT- related systems are distinct at both peripheral (skin) and central (specific brain areas) level (Fig.1).

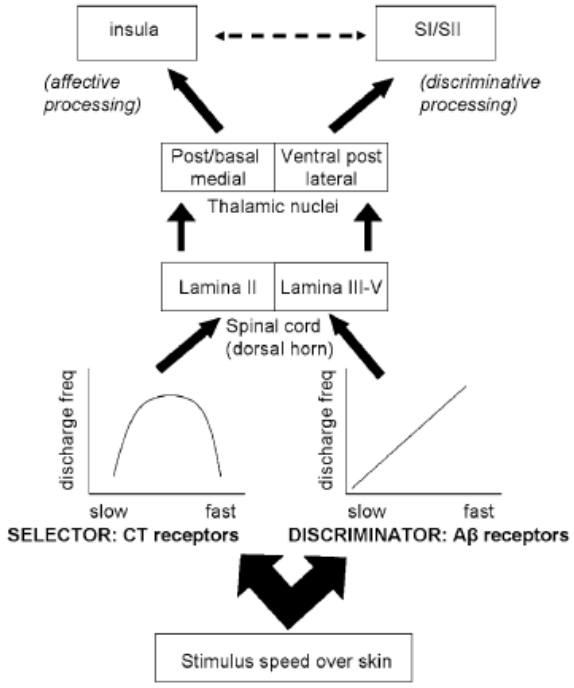


Figure 1. *Schematic model of affective and sensory-discriminative pathways for dynamic touch in hairy skin. These signals follow dissociable pathways to the cortex but probably interact at several levels. Within cortex, reciprocal connections between posterior insula and secondary somatosensory cortex may allow mutual modulation of affective- and sensory-related processing (From Morrison et al., 2010).*

Since the aim of the present study was not related the discriminative aspect of the touch, it will be reported just a brief overview related the mechanism behind the codification of the discriminative component (please see next paragraph). Indeed, since we will focus on the codification of the pleasant touch in non-human primates, therefore on the affiliative component related the CT system, it will be reported a more detailed introduction of CT fibers.

Aβ fibers

The Aβ fibers are myelinated low threshold mechanoreceptive (LTMR) afferents, present in both human and non-human primates skin. The fibers represent the main type of primary sensory neurons that mediate discriminative touch and tactile perception in mammals (Abraira *et al.*, 2013; Fleming and Luo, 2013). Their morphologies and structures have been extensively examined since their discovery in the 1800s, while their physiological properties were firstly identified from the middle of 20th century. The figure 2 shows the different identified mammalian Aβ low threshold mechanoreceptiors

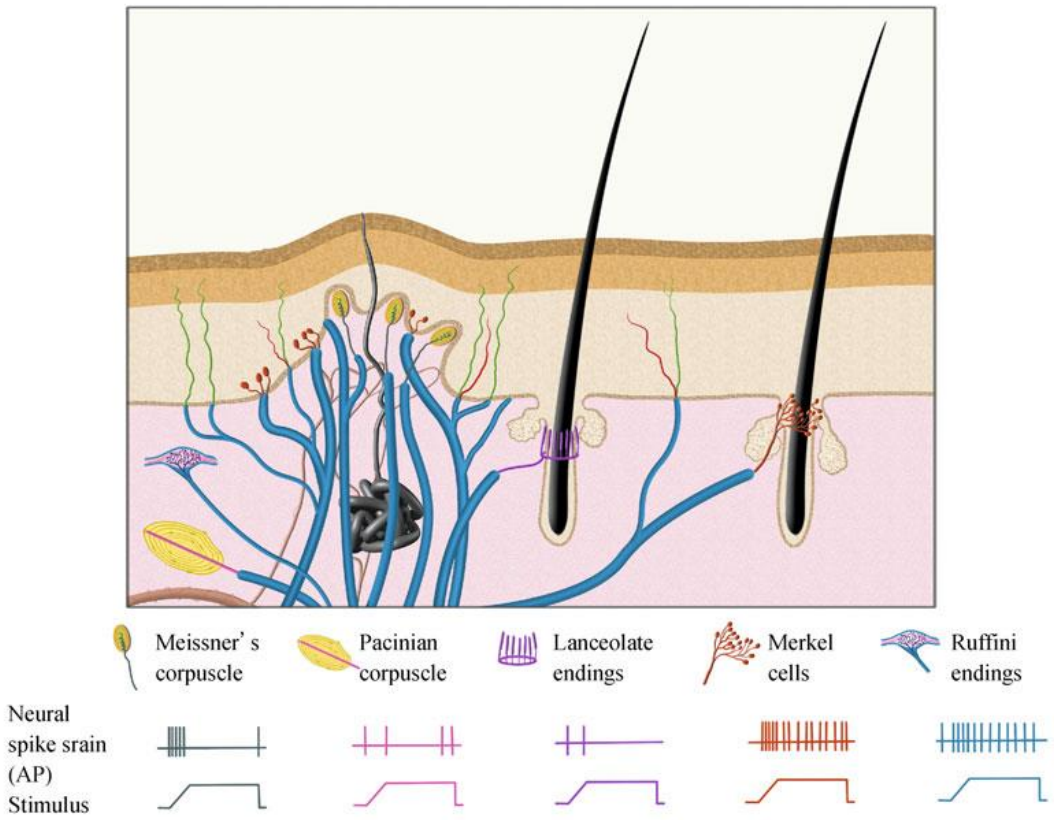


Figure 2. *Mammalian Aβ low-threshold mechanoreceptors. In glabrous skin (left side): Meissner's corpuscles in the dermal papillae of the dermis, Merkel cells in the basal epidermis, Ruffini corpuscles in the dermis, and Pacinian corpuscles in the dermis, deeper than the other mechanosensory end organs. In hairy skin (right side), hair follicles are surrounded by lanceolate endings and Merkel cells.*
The bottom panel shows the neural activity of different types of Aβ low-threshold mechanoreceptors in response to a sustained stimulus. Meissner's corpuscle, Pacinian corpuscle and lanceolate ending mechanoreceptors display rapidly adapting mechanosensitive physiological properties, while Merkel cell and Ruffini corpuscle mechanoreceptors display slowly adapting mechanosensitive properties (From Fleming and Luo, 2013).

These fibers are present in both glabrous and hairy side of the skin and the cell bodies are located in the trigeminal (TGs) and dorsal root ganglia (DRGs). Each neuron grows a single axon that bifurcates shortly after projecting from the cell body, with the peripheral axon innervating mechanosensory end organs and the central projection innervating the spinal cord and brain stem. According to their rates of adaptation to sustained mechanical stimuli, they are classified as either rapidly adapting (RA) or slowly adapting (SA) (Mountcastle, 1957; Iggo, 1985).

The SA discharge continuously to a constant mechanical stimulation, sending information to the brain that the current stimulus is still present on the skin and they can be divided in two types: type I (SAI; Merkel end organs) and type II (SAII; Ruffini end organs). The Merkel end organs are located in the basal epidermis of both glabrous and hairy skin of mammals, while the anatomical location and existence of Ruffini corpuscles between tissues and species is still under debate. For example, they were found on the hairy side of human skin but not of non-human primates skin, where instead sensory endings morphologically similar to them have been identified (Parè *et al.*, 2002).

The RA respond only to changes in mechanical stimuli, at the onset and offset of stimuli, serving as a complementary function to signal that something new is happening on the skin (Johansson and Vallbo, 1979). They can be divided in RA type I (RA; hair units, field units, Meissner end organs), and RA type II (PC; Pacinian end organs). Meissner's corpuscles are primarily located in glabrous skin beneath the epidermis within the dermal papillae. The Pacinian corpuscles are located deep in the dermis, in the subcutaneous fat (Fig.2).

All types of Aβ fibers have large diameter and myelinated axons. Since the myelination of their axons, the conduction velocity of the signal from peripheral skin to central nervous system is very rapid, approximately 50 m/sec. The fast velocity conduction is essential to immediately detect and discriminate localization and all information related the tactile peripheral stimulation. Moreover the fast conduction velocity is also essential for the discriminative aspect of the touch because allow the rapid integration of the passive somatosensory information with other sensory system, such as vision and hearing in order to guide the motor activities and related behaviors (Vallbo *et al.*, 1999; Morrison, 2012).

C-Tactile (CT) fibers

The CT-fibers are low threshold mechanoreceptors found in the hairy skin of various mammals (CT- fibers or C-LTMRs). They were firstly identified in the hairy skin of cats, by the cat saphenous nerve preparation in 1939 (Zotterman, 1939), then in various mammals, including mice, rat, guinea-pig, rabbit, pig and non-human primates (Douglas and Ritchie, 1957; Bessou *et al.* 1971; Iggo and Kornhuber, 1977; Kumazawa and Perl, 1978). Since for long time these fibers were not found neither in the hairy nor in the glabrous skin of humans, it was assumed that they disappeared during evolutionary processes. Therefore, it was supposed that humans did not share this primitive tactile unmyelinated system with others mammals. Just in 1988 (Johansson *et al.*, 1988) it was reported for the first time their presence in the infraorbital nerve of humans, followed by many other studies which underlined that also the hairy side of the arm and the legs were innervated by unmyelinated CT fibers (Nordin, 1990; Morrison, 2012; Vallbo *et al.*, 1995).

Although there is currently no accurate method to assess the innervation density of CT afferents in humans, the microneurography recordings from the lateral antebrachial cutaneous nerve of the forearm underlined that they are encountered as often as Aβ afferents.

The receptive field of CTs are round or oval consisting of one to nine small responsive spots distributed over an area up to 35 mm^2, as illustrated in the figure 3 (Nordin, 1990; Wessberg *et al.* 2003). This receptive field structure is also consistent with animal observations indicating that the receptor is likely of free nerve ending type (Cauna, 1973; Iggo and Kornhuber, 1977; Messlinger, 1996; Liu *et al.*, 2007). Until nowadays, the studies have underscored the absence of a uniform organization of receptive field in the different body parts, even further studies are ongoing to more precise define their organization and innervation though the body. Interestingly CT afferents have never been found in the palm of the hand despite numerous microneurography recordings from this skin area. Hence, it seems reasonable to conclude that they are lacking in the glabrous skin.

Animal and human studies conducted using von Frey monofilaments have determined their physiological properties. Their response is correlated to the force with which an innocuous tactile stimulation is applied, *i.e.* if in the range of 0.22-2.5 mN (milliNewtons), and shows preference for dynamic stimulation across the skin surface (as a caress). The CT-fibers encode vibratory stimuli up to 1 Hz, but a small proportion of the afferents are sensitive to vibration up to 32 Hz. Above 32 Hz CTs only respond with single spikes (Vallbo *et al.*, 1999; Wiklund Fernström *et al.*, 2003).

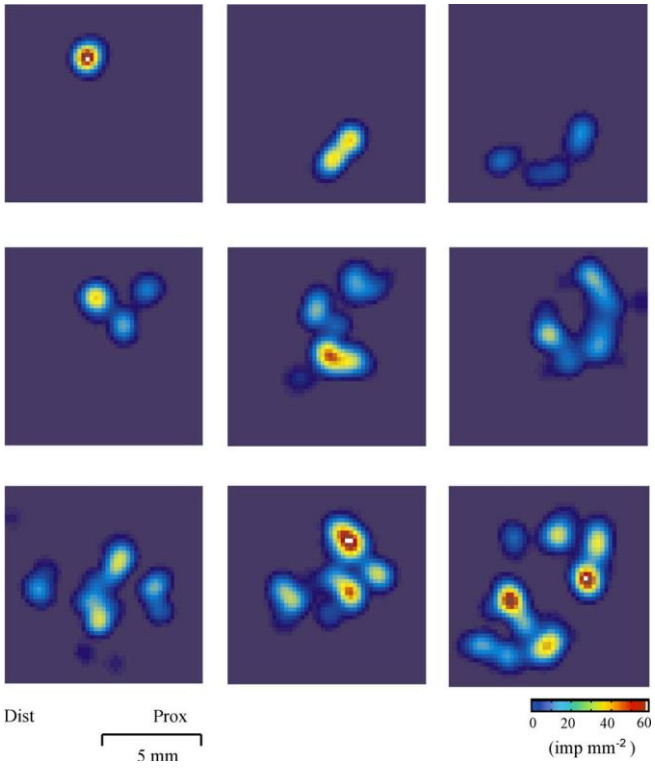


Figure 3. *Field topography of 9 tactile C afferents. The colors represent intensity of afferent firing. From Wessberg et al. (2003).*

When activated, the fibers produce high frequency trains of action potentials with a peak rates of 100 impulses/sec (50-100 impulses/sec). The CTs decrease their firing rate if the velocity of stimulation is lower than 1 cm/sec (about 0.3 cm/sec) or higher than 10 cm/sec (about 30 cm/sec), while they increase the firing rate if the velocity is in the range 1-10 cm/sec. For this property of modulation, their curve of stimulation has the characteristic upside down *U*-firing shape (Fig.1).

With a sustained indentation they respond initially with a high frequency burst of impulses while the rate decreases to zero within 5 sec. The adaptation characteristic (Fig. 4A) of CT afferents is thus intermediate in comparison with the slowly and rapidly adapting myelinated mechanoreceptors.

Another characteristic is the delayed acceleration (Fig. 4B) observed in some units but of unclear functional role. The property determines that 12 sec after the initial phase of adaptation to silence or nearly silence, their firing resumed and successively built up to considerable rates.

Since the degree of myelination determines the velocity of impulse conduction, the CTs being unmyelinated are characterized by slow conduction velocity, in the range 0.6-1.3 m/sec (Vallbo *et al.*, 1999).

At peripheral skin level, it was demonstrated that the CT stimulation evokes a sympathetic skin response. It was investigated by means of recording of the skin resistance changes with

electrodermal recording device, using Ag–AgCl electrodes placed on glabrous skin of the non-dominant hand. The observed sympathetic arousal produced by CT activation is in line with their role in the emotional aspects of touch (Olausson *et al.*, 2008 a, b, c).

Concerning the cortical processing, studies in rats, guinea pigs and monkeys, demonstrated that CT fibers project to the superficial lamina (I and inner lamina II) of the spinal dorsal horn (Kumazawa and Perl, 1978; Light and Perl, 1979; Sugiura *et al.* 1986). At higher levels they project to the ventromedial posterior thalamic relay nucleus (VMpo) that in turn projects to the dorsal posterior insular cortex (Olausson *et al.* 2002 and 2008 a, b, c) with somatotopic organization (Björnsdotter *et al.*, 2009). Human functional magnetic resonance imagin (fMRI) and positron emission tomography (PET) studies have indicated other brain areas could be potentially involved in cortical CT processing: the orbitofrontal cortex (OFC; a key-area for hedonic processing), the postero-superior temporal sulcus, the medial prefrontal cortex (mPFC), the dorso-anterior cingulate cortex, and the pregenual anterior cingulate cortex (pgACC) (Kringelbach and Rolls, 2004; Gordon *et al.* 2013; Lindgren *et al.* 2012; McGlone *et al.* 2007 and 2012; Ellingsen *et al.* 2014).

Their projection from skin to above mentioned brain regions by spinal cord are consistent with the interpretation that the fibers belong to the interoceptive system and that they have pivotal role in the codification of pleasantness and emotional component of the interpersonal touch.

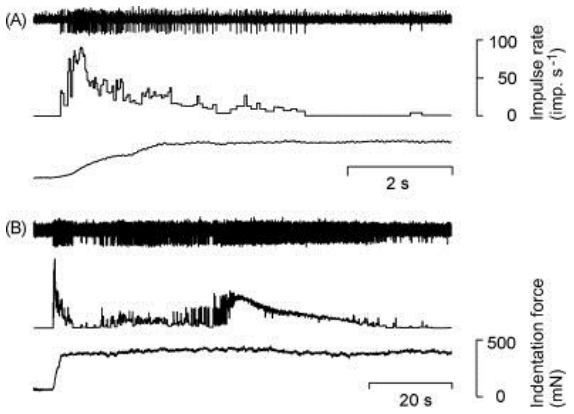


Figure 4. *Microneurography recording of adaptation and delayed acceleration of a single CT fiber. Stimulus was a sustained indentation with a blunt probe.*

A. *Initial part of the test. Here the intermediate adaptation to sustained indentation is illustrated; the impulse rate decreased to zero within 4 s.*

B. *Longer recording of the same test. A delayed acceleration is here illustrated; the impulse rate started to increase again 12 s after the initial phase of adaptation. For a following period of 30 s firing was irregular with recurring short interspike intervals separated by much longer intervals. Then followed a period of more regular firing that climbed to a peak of 40 impulses* s^{-1}*, and then successively declined during a period of 40 s. The subject denied any unique or strange sensation from the skin during the delayed acceleration. (From Vallbo et al., 1999).*

Direct evidence of CTs role in the codification of human interpersonal touch

Direct evidence that could confirm the hypothetical role of CT afferents in the codification of the interpersonal skin-to-skin interaction has been difficult to acquire, since it is not possible to selectively stimulate CT fibers without the simultaneously activation of the Aβ fibers, in healthy people. In fact, CT and Aβ fibers constitute two distinct but not dissociable systems and the unique opportunity to investigate the specific role of CT fibers was given by two different types of patients:

- The two patients with a rare sensory neuropathy (a rare disorder of nerve cell bodies of the large primary sensory neurons) that determined the absence on their skin of myelinated Aβ fibers in large skin areas. Nevertheless, they have intact unmyelinated CT fibers. The two subjects are well described in the literature (Sterman *et al.*, 1980; Cole and Sedgwick, 1992; Forget and Lamarre, 1987), and they are known as GL and IW. They have been extensively studied over the years particularly with regard to motor functions because of their proprioceptive deficit. These patients could detect stimulation of unmyelinated low-threshold mechanoafferents. In fact, in a two-alternative forced choice (2-AFC) test they could detect light touch applied to the forearm skin, where CT are abundant, but failed to detect the same kind of stimuli applied to the glabrous skin of the hand, where CT are lacking. They reported that the sensation was weak and had a pleasant character and had no quality of pain or itch, but their ability to spatially localize CT stimulation is very poor. Moreover, they have difficulties to detect 50 Hz vibratory stimuli which are known to give a poor excitation of CT afferents but a massive activation of Aβ afferents. Finally, fMRI studies showed that selective CT stimulation activated the posterior insular cortex and deactivated both primary and secondary somatosensory cortices (Olausson *et al.* 2002; Wiklund Fernström *et al.*, 2003; Olausson *et al.* 2008a,b,c).
- A unique group of patients with a hereditary disorder associated with a nerve growth factor beta (NGFB) gene mutation, that determined the denervation of unmyelinated skin afferents. Their condition has been classified as hereditary sensory and autonomic neuropathy type V (HSAN-V), and it causes a denervation pattern opposite to that of the subjects GL and IW. The mutation results not in a complete loss of NGF function (Larsson *et al.*, 2009), but in a severe to moderate reduction of unmyelinated C fibers and a moderate reduction of thinly myelinated Aδ fibers, without any neurological or cognitive

abnormalities (Minde *et al.*, 2004). The group of patients exhibiting the HSAN-V mutation is a well-defined population of consanguineous individuals geographically dispersed in the Norbotten region in the north of Sweden, along the Tornea River Valley. Compared with healthy controls subjects, 1) the pleasantness ratings of these patients were lower and the rating pattern across the different velocities (0.3-30 cm/sec) deviated from the typical and normal rating pattern, therefore to the typical inverted U-shaped curve, correlated with CT discharge across velocities (Fig.1); 2) neuroimaging studies revealed that in patients, the stroking speed of 3 cm/sec failed to activate insular cortex and there was no difference in the modulation of insular cortex between the speed of 3 cm/sec (CT-optimal) and the non-optimal velocity of CT activation (30 cm/sec).

CT fibers and brain areas modulation

In all primates the CT afferents project through the lamina I/II of the dorsal horn of the spinal cord to orbitofrontal cortex, limbic system and posterior insular cortex. The CT related pathway from spinal cord to central nervous system has important homeostatic processing properties, which leads to support the idea that these unmyelinated fibers belong to the interoceptive system.

Even if it remains to be explored the exact neuronal network and to which extent the areas are activated, it was widely demonstrated the involvement of the insular cortex, mainly the posterior part.

Functional magnetic resonance studies of patient lacking Aβ fibers (Olausson *et al.*, 2002 and 2010) showed that the selective activation of CT fibers modulates the posterior insula (pIC). On the contrary, in patients with a congenitally reduced density of unmyelinated sensory fibers, the slow, gentle touch fails to activate the pIC, probably because the reduction of the CT afferent inputs to the cortex (Morrison *et al.*, 2011).

Functional magnetic resonance imaging (fMRI) studies on healthy people indicate that the stimulation of CT fibers by gentle touch activates a neuronal network including posterior insular cortex (Björnsdotter *et al.*, 2009).

Taken together, the results obtain from patients and normal people, it could be speculated the pivotal role of the insular cortex in the processing of peripheral information delivery by the CT fibers.

Gordon and colleagues proposed the existence of *"social brain"* involved in processing the affective touch at central nervous system level. They conducted a fMRI study on healthy subjects in order to assess the neural mechanism behind the affective touch decoding. They applied a tactile stimuli consisted of a brush strokes with a velocity of 8 cm/s on the glabrous and hairy skin of the arm, choosing this velocity since in the range of optimal velocity in order to activated the CT fibers. What they found was that the posterior insula was activated during stimulation to both glabrous and hairy skin of the arm, since the region is specific to map the arms. Touch the palm activated the cerebellum and parietal cortex while touch the hairy side of arm activated more the prefrontal cortex and cingulate cortex which are connected with left insula and amygdala. They supposed that the gentle touch CT related and gentle touch not CT fibers related are differently codified at central nervous system level. Since the gentle touch on hairy skin activated the insular cortex and the regions connected with insula, while the touch on glabrous skin activated just the insula but not connected regions, the posterior insula seems implicated in the coding of touch CT related on hairy

skin but not in the touch not related to CT fibers activation, *i.e.* the touch on glabrour skin. These findings suggest that pIC is involved if the affiliative but not discriminative touch codification.

Findings from different approaches including analyses of CT primary afferents in subjects lacking myelinated mechano-afferents, behavioral analysis related the pleasantness of the stimulation, the autonomic effect of the pleasant touch and brain imaging studies, supported the idea that the CT system is not involved in the codification of the discriminative aspect of the touch, instead of the emotional meaning.

CT fibers and autonomic nervous system modulation

The relationship between CT afferents and pleasure has been described from the central nervous system perspective, while the contribution of the peripheral nervous system has received little attention. It was demonstrated in Aβ denervated subjects that the modulation of CT fibers determined a sympathetic skin response. This underlined that CT activation may have autonomic consequences (Olausson *et al.*, 2008c). Löken and colleagues (2009) suggesting for the first time that C-tactile fibers contribute critically to pleasant touch. In fact, in their study, the soft brush stroking on hairy skin was perceived as most pleasant when it was delivered at velocities that were most effective at activating C-tactile afferents (1–10 cm/sec), with a linear correlation between C-tactile impulse frequency and pleasantness ratings. The figure 5 shows the results of their study, underlining that the CT fibers are modulated if the velocity of stimulation is between 1 and 10 cm/sec (Fig. 5 A), that the stimulation applied with that specific velocity is considered pleasant by subjects who received it (Fig. 5 B) and that higher was the firing rate of CT fibers, higher was the pleasantness perception by subjects (Fig. 5 C).

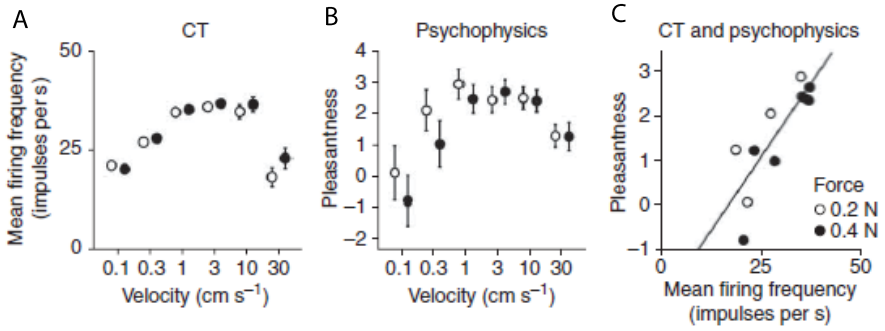


Figure 5. *Neural discharge rate and perception of pleasantness in response to soft brush stroking. A Dots show average discharge rates during brush stroking. B Average ratings of perceived pleasantness in response to soft brush stroking. C Ratings of pleasantness as a function of neural discharge rate in C-tactile afferents. Mean pleasantness ratings are plotted against the corresponding mean firing frequency for each brushing velocity and force (Modified from Loken et al., 2009).*

In contrast, the response of myelinated afferents increased with faster velocities (30 cm/sec) and showed no relationship with pleasantness ratings (Fig. 6 A-D). Moreover, in the palm, which lacks C-tactile afferents, they failed to find any relationship between brush velocity and pleasantness ratings.

This study was the first that demonstrated that the tactile stimulation at the specific velocity of 1-10 cm/sec and pressure (0.2-0.4N) that activate the CT fibers, is perceived as pleasant in normal

subjects. Nevertheless, the authors did not investigated the physiological effects of the stimulation but it could be supposed that the pleasantness reflect the modulation of autonomic system in term of relaxation.

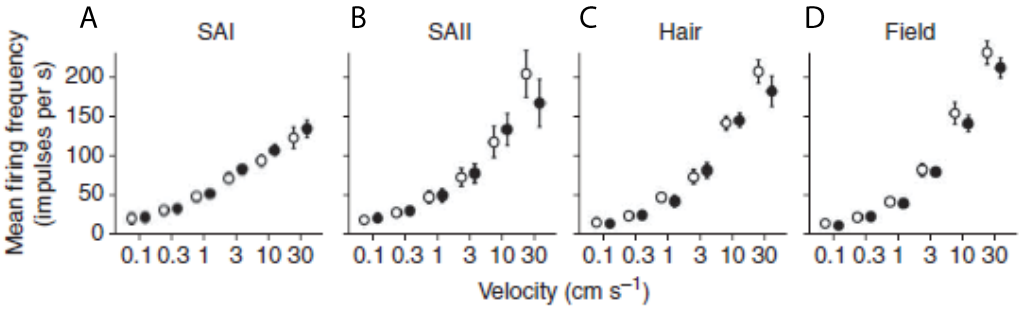


Figure 6. *Neural discharge rate and perception of pleasantness in response to soft brush stroking. Dots show average discharge rates during brush stroking for the different types of mechano-afferents explored with the full range of stimulus velocities. SAI=slowly adapting type I ; SAII= slowly adapting type II; hair= hiar units; field = field units. The mean firing increased monotonically with brushing velocity in all myelinated afferent types (Modified from Loken et al., 2009).*

Recently, it was demonstrated in mice that the activation of the CT fibers determined analgesic effects (Delfini *et al.*, 2013). The observed effects could be considered the indirect evidence of pleasant of the stimulation for the animal, but also the analogous calming effect observed in human studies, therefore the modulation of autonomous nervous system correlated with CT fibers activation. Considering the analgesic effect of CT fibers signaling (Lu and Perl, 2003; Delfini *et al.*, 2013; Vroutou *et al.*, 2013), it was speculated their role in tactile allodynia, a symptom of neuropathic pain where normally innocuous moving tactile stimuli produces pain. Historically this neuropathic pain condition was considering correlated with the Aβ afferents signalings, but it was recently proposed the role of CT system, in particular the in loss of the pain inhibition (Liljencrantz and Olausson, 2014).

Many behavioral human studies demonstrated that the moderate massage and the caress have positive physiological effects toward the vagal activation, both in normal subjects and patients (Lindgren *et al.*, 2010; Diego and Field, 2009; Field, 2014; Tsao, 2007; Billhult *et al.*, 2009; Belinda *et al.*, 2008; Schroeder *et al.*, 2014; Russell *et al.*, 2008). Interestingly, even those studies did not directly measure the CT fibers firing rate, the velocity and the force of the applied massages are in the optimal range that modulate the CT fibers.

It was demonstrated that physical touch such as the caress and moderate massages are employed to reduce the stress of healthy people (Lindgren *et al.*, 2010). In that study, it was reported that just 5 minutes of massage induce the decrement of both the heart rate (HR) lasting for

65 min and the heart rate variability (HRV). Although the reduced heart rate indicates increased parasympathetic activity, both HRV and the high frequency (HF) component decreased during touch massage. The decrement of the HR can be caused by either increased parasympathetic nervous activity or decreased sympathetic nervous activity. However, HRV reflect changes in autonomic activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Therefore, touch massage reduces the heart rate by decreasing sympathetic nervous activity and evoking a compensatory decreased parasympathetic nervous activity in order to maintain autonomic balance. Moreover the authors reported that saliva cortisol and insulin levels decreased significantly after massage, while the serum glucose level remained stable.

In another study on healthy people (Diego and Field, 2014), the moderate pressure massage induced a parasympathetic nervous system response characterized by an increase in HF, suggesting increased vagal efferent activity and a decrease in the LF/HF ratio (low frequency/high frequency component ratio, index of balance between parasympathetic and sympathetic system), suggesting a shift from sympathetic to parasympathetic activity that peaked during the first half of the massage period.

Massage has resulted in positive physiological effects also in preterm infants. In fact it was reported that the massage of preterm neonates 31 weeks old determined increment of their weight gain, reduction of irritability and sleep disturbance, decrement of the HR and increment of the vagal activity (Field *et al.*, 1986).

Massage has also been effective for both acute pain and mood in a study on cancer patients (Kutner *et al.*, 2008; Listing *et al.*, 2009). Interestingly, the massage was more effective than simple-touch for immediate pain relief and mood shifts. Physical touch might also help to improve the wellbeing of cancer patients receiving chemo- and radiotherapy (Russell *et al.*, 2008).

Other painful conditions have benefited from massage including for example chronic pain (Tsao, 2007), post cardiac surgery pain (Cutshall *et al.*, 2010) and Parkinsons (Donoyama and Ohkoshi, 2012).

Massage therapy is also effective in reducing transient anxiety, depression and stress levels in psychiatric inpatients, since it determines a significant reduction in self-reported anxiety, resting heart rate and cortisol levels (Belinda *et al.*, 2008).

Finally it was also demonstrated that massage therapy will improve the leg function and overall quality of life of multiple sclerosis patients, an inflammatory demyelinating disorder of the upper motor neurons illness (Schroeder *et al.*, 2014).

From all above mentioned behavioral, physiological and imaging human studies, it was proposed the Social Touch hypothesis (Olausson *et al.*, 2010) and the Polyvagal theory (Porges, 1995 and 2003 and 2007), concerning the role of CT fibers in the central coding of interpersonal affiliative tactile stimulation and in the modulation of physiological parameters by means of the connection between CT fibers and vagus nerve, respectively (see the relative paragraphs below, for more detailed explanation of both the Social Touch hypothesis and the Polyvagal theory).

Social Touch hypothesis

Based on all above reported studies, Olausson and colleagues (2010) proposed the Social Touch hypothesis also known as Affective Touch hypothesis. This hypothesis posits the CT system as a specific coding channel for gentle, dynamic touch occurring during close affiliative skin to skin social interactions, to support emotional and behavioral responses to interpersonal interactions.

According to this hypothesis, the light touch that we experience as pleasant and that activates the CT fibers is an important part of social interactions and could play a vital role in forming and maintaining social bonds. The CTs may constitute a peripheral support in the emotional, hormonal and behavioral response to that tactile stimulation. From periphery the signal reaches specific brain regions where it will code as affective.

In this kind of somatosensory stimulation the discriminative aspect of touch is not important, but it's essential the affiliative and social communicative aspect of the stimulations. As part of interoceptive system, these fibers contribute to the construction of the sense of self in relation to others. Even the discriminative component is not essential, the combination between CT and Aβ fibers, allow us to build up a complete sense of all tactile component in the hairy skin.

Polyvagal theory

All studies related the autonomic effects of moderate massage support and are supported by the Polyvagal theory (Porges, 1995 and 1997 and 1998 and 2001 and 2003 and 2007). This theory proposed the impact of the CT fibers on the modulation of the vagal nerve towards the unbalance of the autonomic system in favor of parasympathetic branch. The Polyvagal Theory (from greek "polus", "many" and "vagal", "Vagus Nerve") was proposed and developed by Dr. Stephen Porges as an emerging model of neural regulation of the autonomic nervous system. The theory introduced a new perspective relating autonomic function to behavior that included an appreciation of autonomic nervous system as a "system", the identification of neural circuits involved in the regulation of autonomic state, and an interpretation of autonomic reactivity as adaptive within the context of the phylogeny of the vertebrate autonomic nervous system.

According to the polyvagal theory, the vagal system does not represent a unitary dimension instead, there are two vagal motor systems, functionally, neurophysiologically and neuroanatomically distinct and opposing branches of the vagus nerve, the dorsal and the ventral branch. Both branches originate in the medulla. The dorsal branch of the vagus originates in the dorsal motor nucleus and is considered the phylogenetically older branch. This branch

is unmyelinated and exists in most vertebrates and it is also known as the "vegetative vagus" because it is associated with primal survival strategies of primitive vertebrates, reptiles, and amphibians.

With the increase of the neural complexity in mammals, due to phylogenetic development, a more sophisticated system evolved in order to enrich behavioral and affective responses to a complex environment. The ventral branch of the vagus originates in the nucleus ambigus, is myelinated and provides therefore higher control and speed in responding.

The theory articulates three phylogenetic stages of the development of the vertebrate autonomic nervous system, since with increased neural complexity due to phylogenetic development, the organism's behavioral and affective repertoire is enriched (Table 2). There is a phylogenetic shift in the regulation of the heart from endocrine communication, to unmyelinated nerves, and finally to myelinated nerves. Each stage is associated with a distinct autonomic subsystem or circuit that is retained and expressed in mammals. These autonomic subsystems are phylogenetically ordered and behaviorally linked to social communication (*e.g.*, facial expression, vocalization, listening), mobilization (*e.g.*, fight-flight behaviors) and immobilization (*e.g.*, feigning death, vaso-vagal syncope, and behavioral shutdown). The social communication system is dependent upon the functions of the myelinated vagus. Instead, the most phylogenetically primitive component, the immobilization system, is dependent on the unmyelinated or "vegetative" vagus, which is shared with most vertebrates.

Phylogenetic stage	ANS component	Behavioral function	Lower motor neurons
III	Myelinated vagus	Social communication, self-soothing and calming, inhibit sympathetic-adrenal influences	Nucleus ambiguus
II	Sympathetic-adrenal	Mobilization (active avoidance)	Spinal cord
I	Unmyelinated vagus	Immobilization (feigning death, passive avoidance)	Dorsal motor nucleus of the vagus

Table 2. *The three phylogenetic stages of the neural control of the heart proposed by the polyvagal theory (From Porges, 2001).*

Therefore, according to the theory, only in mammals the primary vagal regulation of the heart shifted from the unmyelinated pathways originating in the dorsal motor nucleus of the vagus to include myelinated pathways originating in the nucleus ambigus. The myelinated vagus functions as an active vagal brake in which rapid inhibition and disinhibition of vagal tone to the heart can rapidly mobilize or calm an individual. The myelinated vagus actively inhibits the sympathetic nervous system's influences on the heart and dampens hypothalamic-pituitary adrenal (HPA) axis

activity. Functionally, the vagal brake, by modulating visceral state, enables the individual to rapidly engage and disengage with objects and other individuals and to promote self-soothing behaviors and calm states.

Developmentally, the number of myelinated vagal fibers increases linearly from 24–28 weeks gestation until full-term birth when the number of fibers is comparable to those observed in adolescence (Sachis *et al.*, 1982). In full-term infants, the myelination process is active during the first year of life, particularly during the first three months (Pereyra *et al.*, 1992). Thus, deficits in the regulation of the vagal brake may be causal in deficits in social communication observed early in development. Basically, the expression of social engagement behaviors is dependent upon the regulation of visceral state by the vagal brake. If visceral homeostasis is challenged and the vagal brake is unable to regulate visceral homeostasis, then social engagement behaviors will be minimized. Thus, it is possible that psychiatric disorders, (*e.g.*, autism, schizophrenia, reactive attachment disorder) in which compromised social behaviors are diagnostic features, are associated with neurobiological state regulation strategies

Nevertheless, when the Polyvagal theory was initially presented, knowledge of vagal C-fibers was limited. There was no published demonstration that C-fibers could produce a bradycardia of sufficient magnitude to be clinically relevant. Several plausible explanations were presented in an attempt to understand how the massive bradycardia observed during fetal distress could be mediated via unmyelinated vagal pathways (Reed *et al.*, 1999). During the intervening years, new findings regarding vagal C-fibers are beginning to explain how few C-fibers can produce clinically relevant bradycardia (Porges, 2003 and 2007).

Therefore, the unmyelinated fibers originated in the dorsal root of the medulla is the most primitive branch of the vagal system, associated to the interoceptive, homeostatic system and determined the modulation of vagal system toward its activation. According to the polyvagal theory, the activation of unmyelinated fibers by means of pleasant touch would determined the relaxing effect, by means of decrement of heart rate and increment of heart rate variability therefore the positive modulation of parasympathetic system, though the modulation of the primitive branch of the vagal system. This is in line with the meaning that social interpersonal touch is a primitive tactile stimulation present in many social animals, including non-human primates.

Disadvantage of CTs unmyelinitation

The unmyelinitation of CT axons determined the reduction of the velocity of conduction of the signal. On the contrary, the myelin that covers the axons of Aβ fibers permits to very fast convey signal without any timing delay. The high speed of conduction is necessary in order to immediately identify the features of the touched object.

Therefore, the absence of the myelin covering the axons that determines the slow conduction velocity is not evolutionary advantageous, in term of discriminative aspect of the stimulus. In fact it do not allow us to immediately understand what is the touched object. Nevertheless, the CT system is not designed to detect the localization and the features of objects instead, to provide a degree of pleasant of a specific peripheral tactile stimulation. Recently, Damasio and Carvalho (2013) have reviewed the literature about the feeling and its central decoding. They have hypothesized that the CT system could be evolutionary preserved because processes as the homeostatic ones are not time sensitive, opposite to the touch discrimination. Therefore, the myelin is not essential in this case.

Moreover they supposed that the insula has a modulatory role but not generative and directly remapping roles of the internal body states in relation to interoception. The vagus nerve carries the visceral information, about cardiovascular, respiratory, gastrointestinal and genito-urinary system to the nucleus tractus solitaries of the brainstem. These interoceptive information from brainstem is rostrally remapped in the somatosensory cortices, both primary and secondary somatosensory cortices, and in the insula. Nevertheless, damages in the insula doesn't involved the perception of feeling and interoceptive sensation, while damages to the posterior upper brainstem is associated with coma and vegetative state, in which all feelings are abolished. Therefore, the insula per se it's not necessary to directly coding the interoceptive feeling, as brainstem. Brainstem contains information in implicit form and same information may be explicitly represented in the insula.

Grooming, the affiliative touch of non-human primates

Social grooming is a widespread behavior among mammals, birds and arthropodos. Self-grooming is directed toward the individual's own body, while allogrooming is carried out on others' body parts, inaccessible or invisible to self-grooming. Although the primary biological function of allogrooming is to take care of the body surface of others, many studies demonstrated its social function in many animals (Kimura *et al.*, 1996; Wilkinson, 1986; Böröczky *et al.*, 2013; Crowell-Davis *et al.*, 1986; Tyler, 1972; Rho *et al.*, 2007; Cox, 2012; Radford, 2008) and especially in non-human primates (Spruijt *et al.*, 1992, for a review).

The grooming among non-human primates is characterized by bimanual actions with rhythmic sweeps and plucking movements of the fingernails in precision grip, whilst being directed at addressing skin debris, spots, blemishes, ectoparasites or vegetation trapped in the fur (Tanaka, 1995). Allogrooming is primary carried out to clean others' body parts, inaccessible or invisible to self-grooming (Barton, 1985), and for the control of lice infection (Zamma, 2002). Nevertheless, all non-human primates devote a significant amount of time grooming other individuals, suggesting that there is a reason behind this phenomenon, besides merely the hygiene function (Kummer, 1968; Boccia *et al.*, 1989; Seyfarth, 1977). It has been hypothesized that the allogrooming is the most common affiliative relationship and social strategy to create and maintain relationships and reliable alliances in order to respond collectively to whatever environmental, physical, social, or predatory challenges they may face (Dunbar, 1991). Moreover, it was reported that allogrooming enhance relaxation and feelings of security (Dunbar, 2010), while simultaneously reducing anxiety levels (Schino *et al.*, 1988). These effects were supported by the investigation of physiological parameters, such as heart rate (HR) and cortisol levels. In particular a decrement of the HR when receiving grooming (Boccia, 1989; Aureli *et al.*, 1999), and a reduction of the cortisol levels during both passive (Gust *et al.*, 1993) and active grooming (Shutt *et al.*, 2007) was demonstrated.

In addition to the numerous studies related to allogrooming, also the behavioral and physiological impact of grooming conducted by humans on monkeys or other animals has been explored. For example, the effect of human contact in horses (Lynch *et al.*, 1974), cats (Bernice, 1959), dogs (Fonberg and Kostarczyk, 1980), and in farm animals such as dairy cows (Schmied *et al.*, 2008), cattle and lambs (Tallet *et al.*, 2005) was investigated. These studies underlined that human grooming determined a positive effect in terms of autonomic responses (Lynch *et al.*, 1974) but also in terms of behavior of the animals, for example the interaction and the approach to humans (Bernice, 1959).

Concerning non-human primates, Taira and Rolls (1996) demonstrated that receiving grooming from humans is a positive reinforcement in operant conditioning for Rhesus monkeys. It was also demonstrated that the human grooming to Rhesus monkeys determined the decrement of heart rate and increment of heart rate variability (Grandi and Ishida, 2015). Specifically, the observed physiological effect of autonomous nervous system depend on the stimulated body parts, as demonstrated also in horses (Normando *et al.*, 2002).

Several regions of the central nervous system (amygdala, orbitofrontal cortex, temporal cortex) that receive the projection of CT fibers, have been implicated in the grooming behaviour of monkeys as demonstrated by brain lesion studies. Kling and Steklis (1976), for example, reported that monkeys lesioned in these areas did not show anymore the affiliative behaviours such as grooming. Moreover in our previous experiment (Ishida *et al.*, 2014) we identified single neurons modulated during picking grooming like sensory stimulation.

In a recent review, Dunbar (2010) proposed that the sweeping movements during grooming may activate a class of slow unmyelinated CT fibers. Nevertheless there is no evidence in support of Dunbar's hypothesis related the role of CT fibers during sweeping motions among non-human primates. Therefore:

- Is the velocity of the sweeping among monkeys inside the optimal range of 1-10 cm/sec to activate the CT fibers, as affiliative caress in humans?;
- Are the effects of autonomous nervous system of the sweep similar to those of human caress?;
- Is insular cortex modulated, as during pleasant touch that activates the CT fibers in humans?

Insular cortex, Reil's Island

The insular cortex is a portion of the cerebral cortex situated within the lateral sulcus between the temporal and the frontal lobe. The figure 7 shows its localization in the Rhesus monkey's brain.

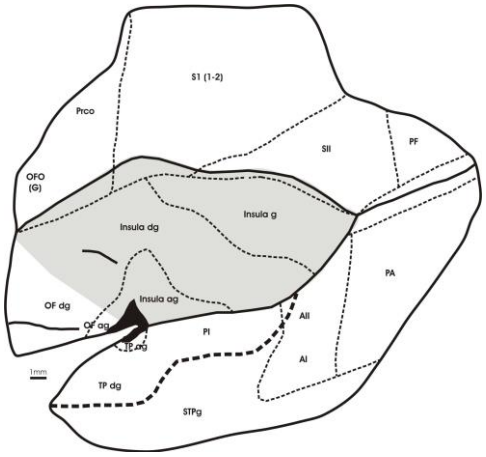


Figure 7. *Localization of insula in the Rhesus monkey's brain (Modified by Mesulam and Mufson, 1982).*

In 1783, Monro published an illustration showing the three gyri of the human insula, visualized after removing the ventral portion of the cerebrum, without define neither describe them. According to the literature, Vicq D'azyr was the first to mention but not to describe this region, in 1786. In his *Traite d'anatomie et de physiologie*, he defined it *"the convolutions situated between the sylvian fissure and the corpus striatum"*. The first to describe the insula was Reil. In 1796 he published the *Exercitationum anatomicarum fasciculus primus de structura nervorum*. Even if this 32-pages Latin treatise is related to the structure of nerves, it also contains a description of the insula. He defined the region *die Insel,* and since that time has been the accepted as the nomenclature for this area, the "insula" or "island of Reil" (Ture *et al.*, 1999). The discovery of Reil was then immortalized in Gray's Anatomy, work of Henry Gray, from the first edition of 1858 until the 39th version. From 1796, for the succeeding 50 years, the insula attracted little attention but from 1860 many studies were designed to define it both anatomically and functionally. At the end of the 19th century, several landmark articles reported in detail the anatomy of the insula and surrounding regions.

Anatomy

Some of the first detail neuroanatomical studies about insula, were published from Robert and Akert in 1963 (Robert and Akert, 1963), Sanides in 1972 (Sanides, 1972), Jones and Burton in 1976 (Jones and Burton, 1976) and Mesulam and Mufson in 1982 (Mesulam and Mufson, 1982 a,b,c and 1984). The most important one was the latter and was published in a three-part-paper. Here they described in detail the architectonic, the afferences and the efferences of the insula. From these studies, many others were conducted. To define the architecture of insula, these authors used different method; Robert and Akert and Jones and Burton analyzed the distribution of perikarya, Sanides the distribution of myelin while Mesulam and Mufson the distribution of perikarya, myelin and acetilcholinesterase (AChE). Despite that, they were agree to divide insula in three regions:

- *Agranular insula (Iag).* Rostro-ventral part of insula, with three agranular layer, the I layer, the II and the III layers are together, the IV layer is absent while the V and the VI layers are together. Here it's present just a layer of myelinic fibers and the highest level of AChE in comparison of the entire insula. The Iag include a part of olfactory prepiriform allocortex (POC).
- *Disgranular insula (Idg).* Dorso-caudal to Iag, this not-totally granular area is the largest in comparison to the other two. The I layer is present as in Iag, the II and the III start to differentiate each other, the IV layer is absent while the V and the VI layers are together. The myelinic fibers are quite poor, if not in the deep layers and AchE level is less than in the Iag.
- *Granular insula (Ig).* Caudal part of insula with the six differentiate granular layers. The II layer is more differentiate from the III in the dorsal than in the ventral part. In comparison to Iag and Idg, here there's an increment of myelinic fibers while the AChE is quite absent.

Recentely, Evrard and colleagues (Evrard *et al.*, 2014) subdivided the 3 already well identified regions, granular disgranular and agranular ones in 4,4 and 7 sub-regions, respectively (Fig. 8). They analyzed 10 long tail macacque monkey brains with the Nissl and Gallyas anatomical techniques.

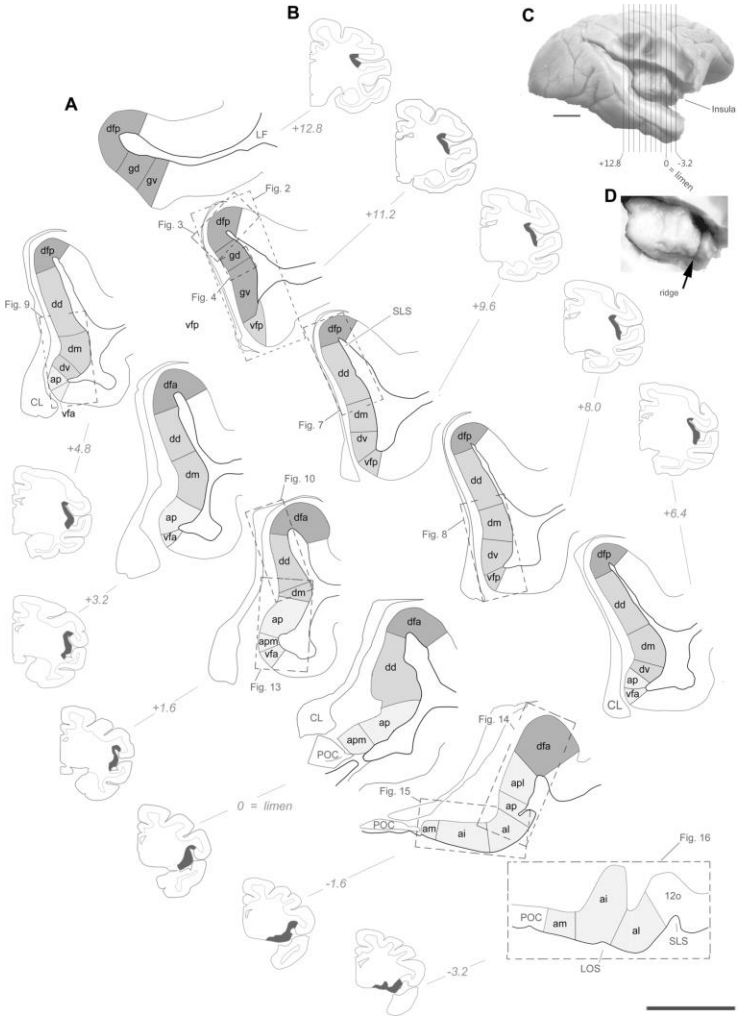


Figure 8. *Location of the 15 distinct areas in consecutive coronal sections from the right hemisphere of one case of the study. Illustration of the topographical localization of the 15 distinct architectonic areas of the insular cortex in one set of drawings of 11 coronal sections from the right hemisphere from one representative case, cm6R.* ***A.*** *Drawing of the 15 architectonic areas and borders. The granular, dysgranular and agranular areas are distinguished by darker, intermediate and light gray tones, respectively. Scale bar = 2.5 mm.* ***B.*** *Low magnification and simplified drawing of the entire coronal section from which the drawings in panel C were made. The extent of insular cortex shown in panel C is represented by the darkened areas. The anteroposterior position of the section is indicated on the right or left of the section using the limen insulae as the zero.* ***C.*** *Approximate anteroposterior position of the 11 coronal sections juxtaposed on a lateral view photograph of a different brain in which the insula was exposed by dissection.* ***D.*** *Higher magnification of the same lateral view showing the anterior vertical ridge of the insula (arrow). 174x238mm (300 x 300 DPI) (From Evrard et al., 2014).*

Anatomo-functional connections

Applying various methods, it has been demonstrated that the insula is connected with frontal, parietal and temporal lobes, the cingulate gyrus, the basal nuclei (the tail of the caudate nucleus, the putamen, and the claustrum), the amygdaloid body and other limbic structures, and the dorsal thalamus. These connections suggest that the insula may function as a visceral sensory, visceral motor, supplementary motor, and vestibular organ and may also be related to language. The figures 9, 10, 11 and 12 resume the insular connections (Augustine, 1993).

Anatomo-functional cortical and subcortical connections

Pribram (Pribam *et al.*, 1950 and 1952) had applied a stricnine-based neurography to identify specific cortico-cortical pattern among insula, orbital regions, temporo-polar, opercular and supra-temporal areas. Even if this method is not reliable because of its intrinsic problems, the data obtained are confirmed by more recent studies. These studies underlined the connection between insula and inferior prefrontal convexity, orbitofrontal cortex, area 6, pre-central gyrus, primary somatosensory cortex, inferior parietal lobe and fronto-parietal operculum. Moreover they identified by the injection of anterograde and retrograde tracers (HRP and TAA) the cortical afferences and efferences of the different single areas and sub-regions of insula; even if the three insular regions are connected with the cingulate cortex and with the temporal, frontal and parietal lobes, there's a different density of connections for each one, thus the division based on granularity reflects also the weight and the importance of each connection. The strongest and most important cortical connections could be summarized as follows;

- *Agranular anterior insula (Iag)* has the strongest connections with the frontal lobe, in comparison to the others insular regions. In detail, the connections are present in lateral area of prefrontal lobe, the orbitofrontal region, orbitofrontal operculum (OFO), orbitofrontal granular, agranular and disgranular regions (PrCO) and areas 46 and 12, ventral premotor area and pre-supplementary motor area (F6, pre-SMA). The connections with temporal lobe concern the agranular and disgranular portions, and the anterior parts of the superior temporal sulcus (STS). The connections with caudal temporal lobe regions are totally absent. The Iag is poorly connected with the parietal lobe and cingulate cortex. In the first, there are connections with the area of mouth representation in the primary somatosensory cortex (SI), while in the latter, with the anterior part (ACC). Finally, there is a strong internal connection between Iag and POC. For what concerns thalamic connections, the Iag is connected with the parvi-cellular portion of the

ventropostero-medial nucleus (VPMpc), Pulvinar and with the para-lamellar division of the dorsal medial complex (MD). The thalamic nuclei-Insula connections are rostro-caudally organized in relation to the afference of autonomic nervous system. In fact the Iag is connected with VPMpc and this one is connected with the solitary tract nucleus (NTS). By the circuit Iag-VPMpc-NTS, the anterior insula is implicated in the modulation of parasympathetic nervous system. Moreover, by the connection with MD complex, the Iag seems to be involved in cognitive and emotional functions.

- *Disgranular insula (Idg)* has the same connections as the Iag, with the frontal lobe, except with OFO and PrCO. The connections with the temporal lobe concern the primary and secondary auditory cortices (AI and AII, respectively), PA, RI and the anterior portion of STS. The Idg is strongly connected with the parietal lobe, in particular with SI, SII, the superior parietal lobule (PE) and with the inferior rostral parietal lobule (PF). Poor connections are described with cingulate cortex, mainly just with areas 23 and 24. For what concerns thalamic connections, the Idg is connected, as the Iag, with the parvi-cellular portion of the ventro-postero-medial nucleus (VPMpc) and Pulvinar.

- *Granular posterior insula (Ig)* has very poor connections with the frontal lobe. The portions of temporal lobe connected with the Ig are TPag, PI, TPg, AI, AII and STS. Connections with PA and RI are poor and with POC they are absent. The connections with the parietal lobe concern its inferior portions, thus PF,PFG and PG, the opercular portions of SI and SII and the superior part of PE. Moreover there are connections with cingulate cortex, mainly with areas 23 and 24. For what concerns thalamic connections, the Ig is connected with the infero-posterior ventral nucleus (VPI), medial genicolatus nucleus, supra-genicolatus nucleus and, as Iag and Idg, with the Pulvinar. The posterior insula, by the connection with VP, which is in turn connected with the lamina I of spinal cord, seems to be involved in the modulation of the sympathetic nervous system.

Instead, the strongest and most important subcortical connections could be summarized as follows;

- *Amygdala.* Anterior and medial amygdala and the magnocellular basal, medial basal and lateral nuclei, are connected with Iag and Idg. The basolateral nuclei are the biggest and phylogenetically the most recent among the central nuclei and show connections with many different cortical polisensory and frontal areas. The central nuclei regulate many different autonomic, neuroendocrine and behavioral responses through the connections with hypothalamus, reticular formation of spinal cord and different regions of brain stem. These

nuclei modulate the release of dopamine, serotonine, noradrenaline and acetylcholine. The direct connection of insula with them is in favor of the autonomic control by anterior and medial insula.

- *Hypothalamus*. The neuroanatomical studies of Ongur and Price at the end of 1990 (Ongur and Price, 1998), demonstrated two types of connections. The first one concerns the rostral part of hypothalamus with a small agranular part of rostral insula located in the orbitofrontal region, rostral to the opening of the lateral sulcus. The network involving this small rostral agranular insula, hypothalamus and prefrontal regions, is the so called "medial prefrontal network" (Charmichael and Price 1996; Ongur and Price 1998). It is implicated in the control of autonomic and homeostatic regulations by mediation of the brain stem nuclei. The second one creates the so called "orbital prefrontal network" and concerns the connection of a small caudolateral portion of hypothalamus with Iag together with the connection with the posterior orbitofrontal area. This network receives, process and sends sensory information to the "medial prefrontal network", which in turn sends its efference to the basal nuclei of amygdala and to the substantia nigra. Moreover the "orbital prefrontal network" is connected with the caudal part of hypothalamus, that is in turn connected with brain stem nuclei, so it's involved in the regulation of parasympathetic nervous system (in particular dorsal vagal nerve and nucleus ambiguous). In turn, this control is done also by the Iag.

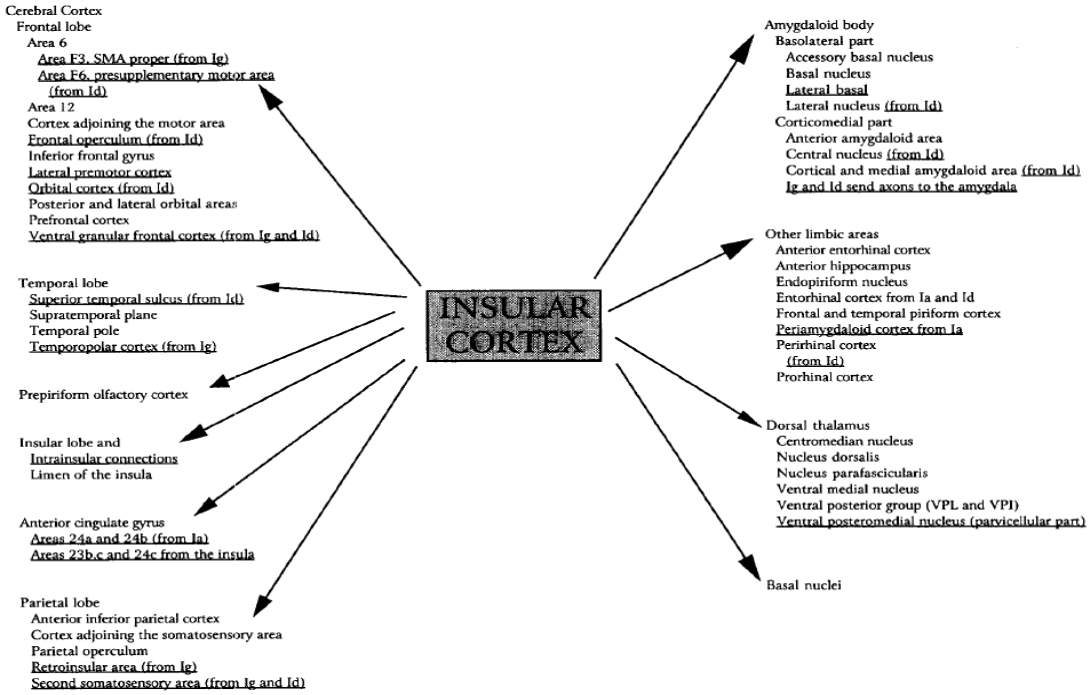


Figure 10. *Efferent insular connections (From Augustine, 1993).*

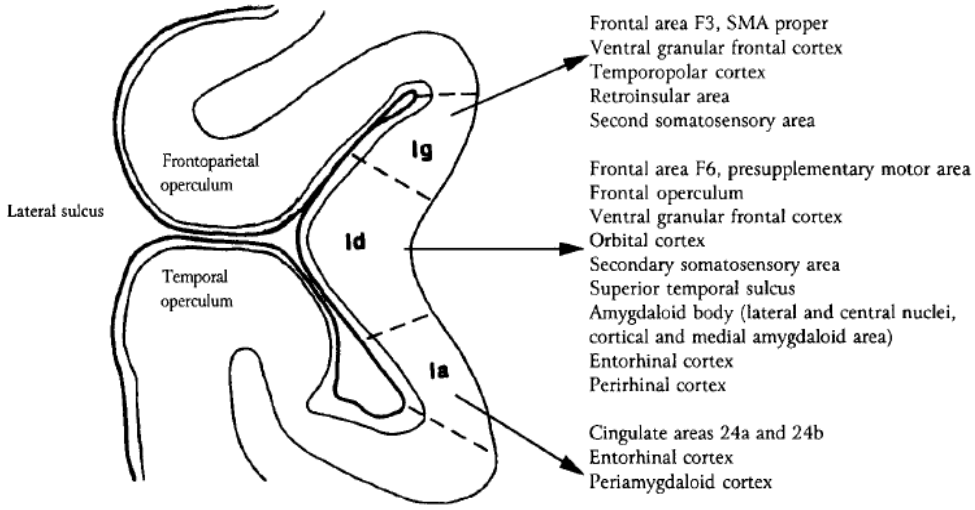


Figure 10. *Efferent insular projections from each cytoarchitectonic portions. Frontal section (From Augustine, 1993).*

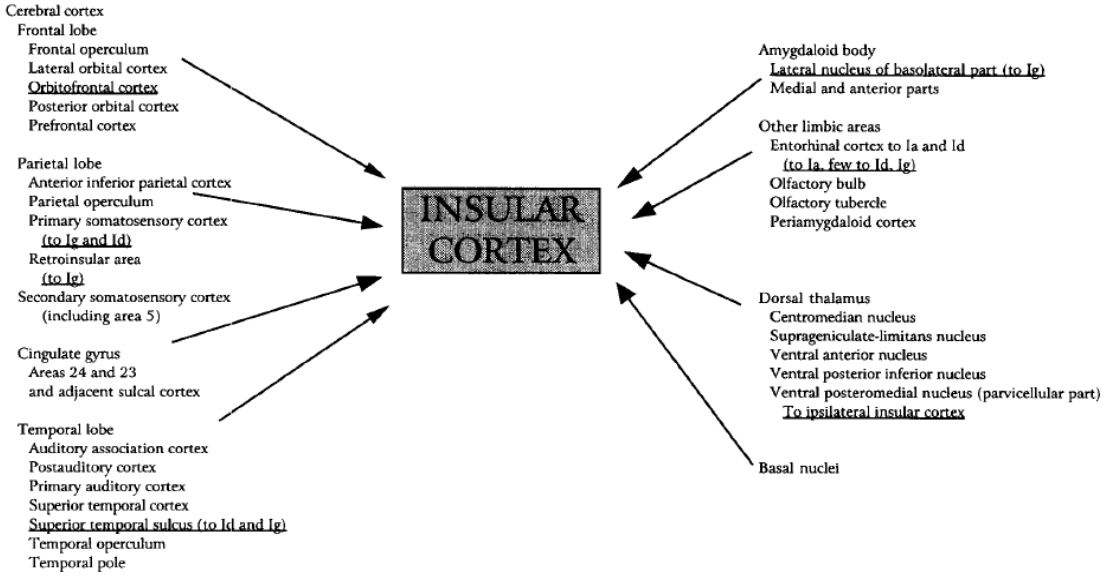


Figure 12. *Afferent insular connections (From Augustine, 1993).*

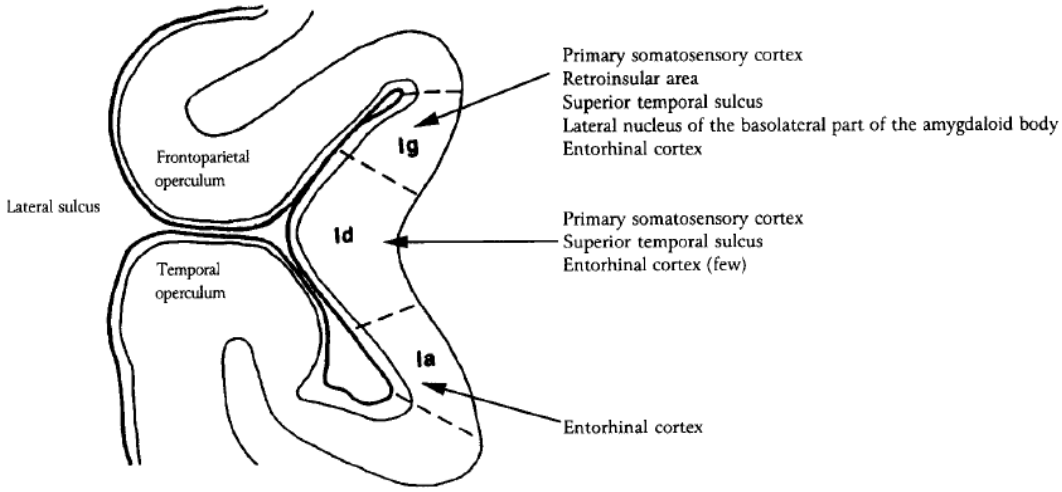


Figure 12. *Afferent insular connections to each cytoarchitectonic portions. Frontal section (From Augustine, 1993).*

Physiological properties of macaque monkeys' Insular cortex

Most of the studies conducted in order to investigate the functional roles of the insula are related to fMRI human studies and from studies related to people with insular damage. What about the roles and the single neuron modulation of insula in non-human primates? The literature is poor of electrophysiological studies concerning the insular cortex of non-human primates and moreover the older ones were conducted on anesthetized animals. Studies with anesthetized monkeys have the strong limitation to not allow the investigation of motor, visual and behavioral responses of recorded neurons. The data can be obtained just from passive proprioceptive and somatosensory stimulation.

The main reasons of this lack of studies related to single neuron activity are the depth of region and its high vascularization. The single electrode is the only technique that allow the recording and discrimination of a single neuron modulation but it is an invasive technique. For this reasons it can be dangerous to reach insula without determine any damage, using a single electrode considering its depth and vascularization. Even the difficulties it is possible to reach this region with single electrode in awake monkey, as some studies demonstrated.

Moreover, recently two electrical stimulation studies were performed. Even if the stimulated area with a single electrode is very small, the current can reach the neighbors areas. Therefore, the visible behavior reaction of monkey after the stimulation, could be cause to their activation by means of connection with the directly stimulated region.

The results from the single unit recording are related to a specific neuron of a specific area, while the results of electrical stimulation should be interpreted as related to the interested region but not as the properties of single neurons.

The two methods can be comparable each others but they give two different kind of results and one cannot substitutes the other one, instead they can be consider complementary and interconnected. The two reported studies related to the electrical stimulation of insula, are the most recent physiological works of non-human primates' insular cortex.

Single unit recording studies

In 1990 Yaxley and colleagues (Yaxley *et al.*, 1990) tested the gustatory responses of different central nervous system areas, including rostro-dorsal insula, by single unit recording technique, in macaque monkeys. They used 33 stimuli (grape juice, water and different combinations of NaCl, glucose, HCl and QHCl). The results showed that the anterior insular neurons activity was selectively depended on the type of the stimulation taste. They concluded that a possible role of insula should to be a primary gustatory cortex.

A weak point of this interpretation is that, since the motor, visual and somatosensory properties were not tested in this study, it is not possible to surely assume that there's no integration or influence of these latter with gustatory responses.

The study conducted by Verhagen and colleagues in 2004 (Verhagen *et al.*, 2004), showed that the neurons in anterior insula were modulated not just by the taste of stimuli but also by the physic intraoral characteristic of these, such as viscosity, texture and temperature. They found both unimodal and multimodal type of neurons, but in any case there was not integration with visual, olfactory and auditory stimuli.

Remedios and colleagues (2008) recorded auditory related responses. They tested different kind of sounds and compared the neuronal responses to those in the auditory cortex. The insular neurons showed selectivity for naturalistic sounds, especially for the conspecific vocalizations. Moreover, neurons were modulated on the basis of type of vocalizations and this suggests the hypothesis of an emotional modulation of neuronal activity.

Schneider and colleagues (1993) observed that neurons responded to superficial touch over the whole body or to the intraoral stimulation and out of 159 tested neurons, just 7 were bimodal visuo-tactile and only 1 was bimodal audio-tactile, but there was not gustatory-responsive neurons. The tactile receptive fields were very large. Based on their results, there is a somatotopical organization from rostro-ventral portion to dorso-caudal one, even if there is not a sharp organization as in the somatosensory cortices. In the most anterior part there are bilateral trigeminal representation while in posterior part there are hand-finger tactile representations, followed by arm, upper part first and lower part then of the trunk, and finally lower limbs representations. Moreover, the posterior insula neurons responded also to the nociceptive stimuli apply to large body parts. From this observation it's possible to postulate that the response is determine by the projections of C fibers, unmyelinated afferents at skin level activated during nociceptive stimulation al level of the skin (Fig. 13).

Recently Mizuhiki *et al.* (2012) reported the reward related activity of anterior insular neurons of monkeys.

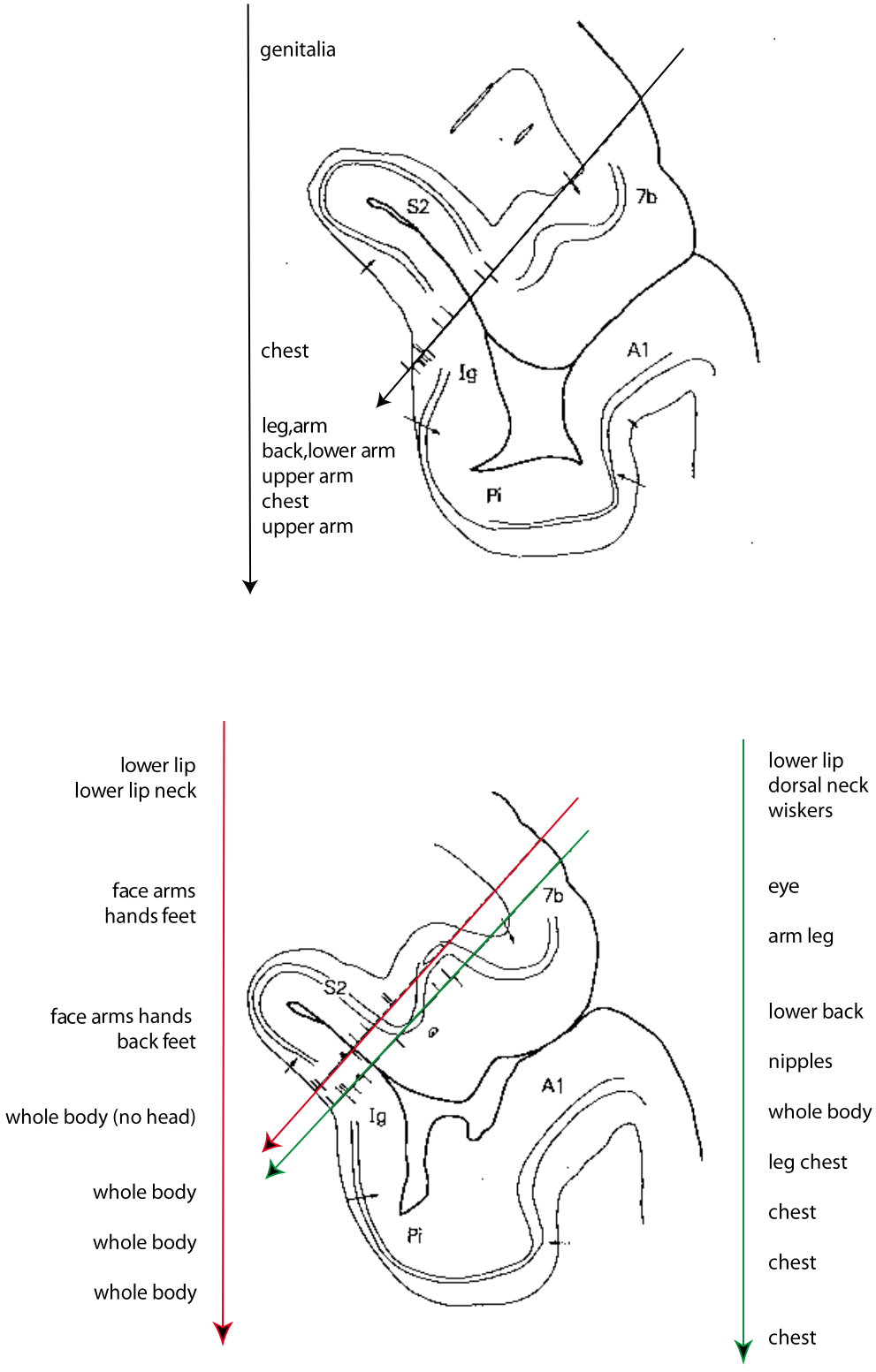


Figure 13. *Three representative penetrations through the insula. In each track the responsive units from single unit recordings, are reported and indicate by a mark. The arrows indicate the direction of penetration (Modified from Schneider et al., 1993).*

Electrical stimulation studies

The two most recent electrophysiological studies concerning the intra-cortical stimulation (ICMS) of insula in awake macaque monkeys, was conducted by Caruana, Jezzini and colleagues in 2011 and 2012 respectively (Caruana *et al.*, 2011; Jezzini *et al.*, 2012). In 2011, Caruana and colleagues demonstrated by an electrical stimulation experiment that different region of Rhesus macaque monkey insular cortex are involved in the modulation of different emotions, in particular disgust and affiliative behavior. They performed an intracortical micro-stimulation (ICMS) in two adult behaving monkeys applied trains of biphasic pulses with duration of 3 seconds and intensity of 4 mA. The disgust-related responses were obtained after the ICMS of the rostral agranular and disgranular insula (Fig.14). The ICMS of a large region of ventral disgranular insula elicited the affiliative like behavior (Fig.15).

The disgust response was characterized by facial grimace or by the refuse of the food if monkey was eating or bringing the food to the mouth. The affiliative responses were characterized by the lip smacking done by the monkey in front of experimenter, in the presence of eye-contact with him. In the absence of eye contact, the ICMS did not evoked the lip smacking, even the eye contact with the experimenter. In the presence of eye contact but absence of ICMS, monkey did not show this kind of affiliative behavior. The authors reported that occasionally monkeys showed the chewing movements after ICMS. These movements are considered to be part of the monkey behavioral repertoire associate with lip smacking behavior. The results of Caruana *et al.* (2011) studies were in agreement with early studies of ICMS of insula (Hoffman and Rasmussen, 1952; Frontera, 1956; Shower and Lauer, 1961). The novelty of the work, was that the ICMS was applied in behaving monkeys while the others studies were carried out in anesthetized monkeys, so the motor responses such as lip smacking can be correlated with specific emotional responses, such as the affiliative ones. So for the first time, it was demonstrated that monkey's insular cortex modulated the social communicative behavior and the emotional responses in relation to the external environment.

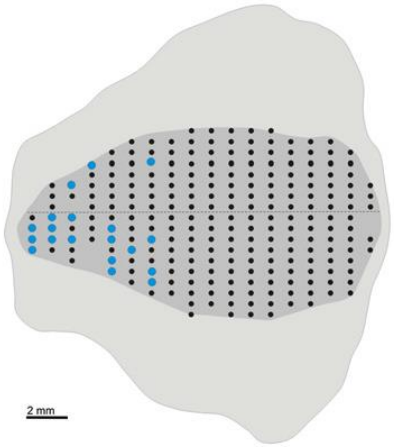


Figure 14. *Unfolded view of the lateral sulcus. Dark grey area above the horizontal line indicates the dorsal insula, while the dark grey below the horizontal line indicates the ventral insula. The light grey area indicates the frontoparietal and temporal opercula of the lateral sulcus. The blue dots indicate the sites where ICMS evoked the disgust-related responses while the black ones where ICMS didn't evoked any kind of responses (From Caruana et al., 2011).*

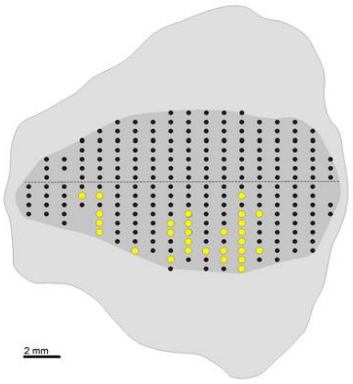


Figure 15. *Unfolded view of the lateral sulcus. Dark grey area above the horizontal line indicates the dorsal insula, while the dark grey below the horizontal line indicates the ventral insula. The light grey area indicates the frontoparietal and temporal opercula of the lateral sulcus. The yellow dots indicate the sites where ICMS evoked the affiliative like behaviour as responses while the black ones where ICMS didn't evoked any kind of responses (From Caruana et al., 2011).*

In 2012 Jezzini and colleagues published a work related to the functional organization of the insular cortex of macacque monkey, determined by the ICMS. They revealed the presence of two fields in the insula;

- ✓ Dorso-caudal portion of insula, related to the sensorimotor functions; the neuronal discharge was related to the mouth, face, hand or limbs (upper or lower ones) movements, eye blinking, grasping related, upper limbs goal related movements directed to the mouth or others body part, visual inspections of the hand, compulsive motions to withdraw something from the mouth and also loss of muscular tone. The figure 16 A shows the sites where these responses were evoked.
- ✓ Rostro-dorsal area, related to the ingestive behaviour consisted of chewing, mouthing, licking, repetitive protusion of tongue or occasionally swallowing. Ventrally to this area, the ICMS evoked a disgust behaviours, such as refuse of food, associate to the facial grimace, the typical facial expression related to food refusing. The ICMS of the most rostral of the Sylvian upper bank evoked the inhibition of any arm movements performed by the monkey. The figure 16 B shows the sites where these responses were evoked.
- ✓ Middle part of the ventral insula, related to the affiliative behavior, tipically lip smacking in case of the eye-contact with the experimenter, as also Caruana reported (Caruana *et al.*, 2011). The figure 16 C shows the sites where lip smacking response was evoked.

Moreover, Jezzini reported also that the ICMS of ventral insula and the lower bank of the Sylvian fissure evoked responses that they defined miscellaneous (Fig.16 D) and included compulsive repetitive movements of the hands or feet, postural adjustment, discomfort reactions (ranged from small postural adjustments to psychomotor agitation), facial grimace of distress often accompanied by psychomotor agitation, rotation of the trunk to the contralateral space and simultaneously, a shift of gaze in the same direction, twitches of chest muscles often followed by general psychomotor agitation, tremors diffused to the limbs or even the whole body but without any somatotopic arrangement. Finally, there were some unresponsive sites in the entire insula, mainly concentrated in the lower Sylvian bank and the ventral insula, as shown by the black dots of figure 16 E. As shown in the figure 16 F, the majority of neuronal response were the miscellaneous ones (32,7%), followed by the sensorimotor and unresponsive response (each of 25,6%).

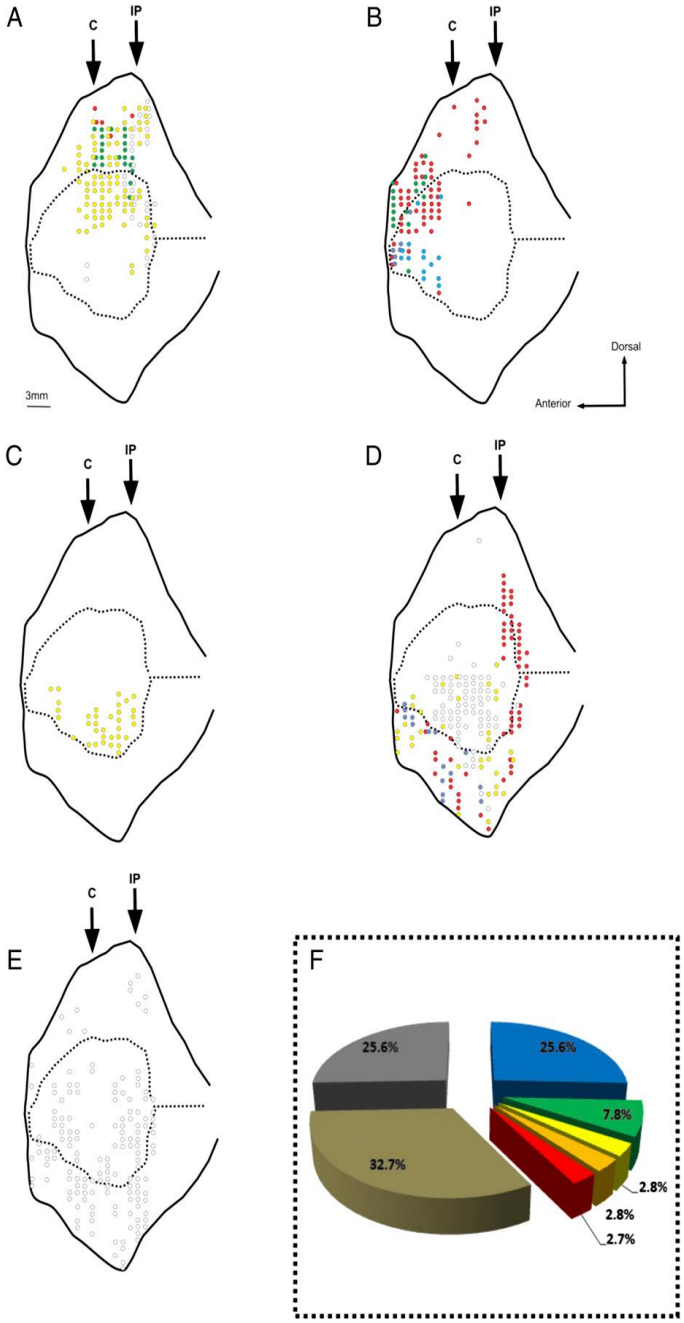


Figure 16. *Unfolded view of the lateral sulcus of the left hemisphere of M1 depicting its upper and lower banks and the insula. Each dot indicates the entrance point of the electrode. Black arrows indicate the antero-posterior (AP) position of the central sulcus (C) and the intraparietal sulcus (IP). In all penetrations, several sites were stimulated every 500 mm below the entrance point and above the exit point.*
A *Posterior dorsal field. Red dots, mouth movements; yellow dots, hand movements; green dots, face movements; grey dots, lower and upper limbs.*
B *Anterior field. Red dots, ingestive behavior; blue dots, disgust behavior; green dots, movement inhibition.*
C *Ventral field. Yellow dots, affiliative behavior.*
D *Miscellaneous responses. Grey dots, discomfort reactions; purple dots, gaze–trunk contralateral displacement; yellow dots, twitch of the chest; red dots, tremors.*
E *Unresponsive sites.*
F *Percentage of sites per each category. Blue, sensorimotor sites; green, ingestive sites; yellow, disgust sites; orange, affiliative sites; red, movement inhibition sites; tan, miscellaneous responses sites; grey, unresponsive sites.*

Phylogeny and phylogenetic hypothesis

According to Sanides' hypothesis (Sanides, 1970) the neocortex originates from gradual development of the allocortex. The allocortex consists of two proto-cortices; the paleocortex (amygdala) and the archicortex (hippocampus). Phylogenetically, the development of these two cortices, precedes the development of the neocortex, and they are present from the origin of Amphibia. Just from the origin of reptiles, appeared a pery-allocortex, consisting of two cortex; a pery-paleocortex (anterior insula) and a pery-archycortex (cingulate cortex). So the anterior insula seems to be the most archaic region of cortex. In macaque monkeys, the pery-paleocortex expands laterally while the pery-archycortex expands medially and both meet at the level of the convexity of sulcus of frontal lobe. The Sanides' hypothesis reflectes the architectonic development of insula, reported by Mesulam and Mufson (Mesulam and Mufson, 1982 a,b,c). They reported that the paralimbic cortex (anterior insula, temporal lobe and orbital cortex) develops in a concentric circles way with the agranular ring, consisting of agranular insula (Iag), agranular orbitofrontal area (OFag) and agranular temporal area (TPag), as the center; the second ring consists of disgranular insula (Idg), disgranular orbitofrontal area (OFdg), situated rostrally to Idg, the agranular/disgranular frontal operculum (PrCO), dorsally to Idg and the two disgranular temporal areas (TPdg and PI) in the ventral part of Idg. The granular ring as the third (Ig, OFO, STP, AI, parietal operculum), and finally an external koniocortex (Retro-insula, AI and BA3).

In 1920 the insular cortex was defined by Ariens Kappers (Ariens Kappers, 1920-1921; Ariens Kappers *et al.*,1936) as a cerebral cortex in proximity to the claustrum and inside the operculum and proposed the phylogenetic hypothesis of "gradual opercularitation of insula". Just three orders of Mammalia have this cortex: ungulates, carnivores and primates, even if the operculum is not totally complete and develop neither in the ungulates, nor in the carnivores, while it's present in primates. Moreover in these Mammalia there is a progressive growth from superior primates to humans. In macaque monkeys, the operculum includes the insula and adjacent rostral and ventro-caudal regions, but the central sulcus of insula is not present. The sulcus compares first in the superior primates.

Finally, the architectonic studies of Jacob (Jacobs *et al.*, 1984) and Manger (Manger *et al.*, 1998) demonstrated that dolphins have architectonic structure of insula in continuity with that of primates.

Onthogeny and onthogenetic hypothesis

The phylogenetic hypothesis concerning the gradual opercularization of the insula is valid also from the onthogenetic point of view. In 1912 Streeter (Streeter, 1912) reported that during human pregnancy, insular cortex is the first cortex of central nervous system to develop and differentiate. In 2007 Afif and colleagues (Afif *et al.*, 2007) chronologically divided the development of central nervous system during human pregnancy in five steps and showed that the process of opercularization of the insula starts in the third step, from the 20th to the 22nd week, and parallel the central sulcus of insula becoming visible. The opercularization is faster in right hemisphere than in the left one but in both it starts from parietal and temporal lobes and finishes in the frontal lobe. The posterior insula closes from the 24th and the 26th week while the opercularization completely finishes during the 27th and 28th week for right and left hemisphere, respectively. According to this view, the opercularization of insula takes place during the later stages of development of the central nervous system. Moreover the process is chronologically different in the two hemispheres, and for this reason one could speculate the presence of different functional aspects of right and left insula, but this aspect is still under debate, despite some human studies about it.

Kinematic analysis of real sweeping during allogrooming among free ranging monkey: Study 1

(Grandi LC, Roda' F, Ishida H. (2015). Physiological effect of sweeping grooming movements in macaque monkey: preliminary data. Journal of Primatology. 4: 126. doi:10.4172/2167-6801.1000126)

Aim of the study

The aim of the present study was to investigate for the first time in macaque monkey if the velocity of the sweeping movement during conspecific allogrooming would be within the range of optimal velocities required to activate the CT fibers in human. For this purpose, we evaluated the velocity of sweeping motion during real allogrooming among free ranging Rhesus monkey, by means of a kinematic analysis.

Material and Method

In order to evaluate the velocity of the sweeping movements during grooming among monkeys, we analyzed 375 videotapes (25 frames/sec) selected and extracted from videos recorded at the National Institutes of Health (NIH, Poolesville, Maryland) by Dr. Francesca Rodà. The videos were recorded inside a group of semi free-ranging Rhesus monkeys (*Macaca mulatta*). The group consisted of 99 monkeys, in detail 34 adult females, 4 adult males, 45 juveniles and 16 babies.

For each video, the period of time from the first contact between the hand of the agent monkey and the fur of the recipient one, to the end of the sweeping motion was recorded. This analysis was performed by means of dedicated software (Tracker2). For each video 1) it was calculated the trajectory from the first hand-body contact time to the last one, in pixel; 2) the pixel values were converted in centimeter, by means of conversion factor known the resolution (dpi) of the PC monitor (96 dpi) and finally 3) it was calculated the velocity in cm/sec. The 375 videos comprised of the following actions: 130 sweeps on the back, 2 on the belly, 16 on the chest, 150 on the head, 68 on the side of body and 9 on the tail. In order to investigate possible differences among the velocities and body parts, we performed the Mann–Whitney U Test ($p < .05$). We selected a non-parametric analysis because the number of samples was different for each body part.

Result

The 375 videos highlighting the sweeping movement of monkeys during allogrooming included 130 sweeps on the back, 2 on the belly, 16 on the chest, 150 on the head, 68 on the side of body and 9 on the tail. In detail, the velocity on the back was 11.63 cm/sec, on the belly was 7.59 cm/sec, on the head was 9.95 cm/sec, on the side of body was 8.69 cm/sec while on the tail was 7.88 cm/sec. The statistical analysis of these videos underlined that the velocities did not differ each other's (Fig. S1.1). The mean value of velocity, independently from the body part, was 9.31 cm/sec. Considering the absence of any statistical difference, we could consider that the mean velocity of the sweeping movement among monkeys is within the range of the optimal velocity to activate the CT fibers in humans, approximately 10 cm/sec.

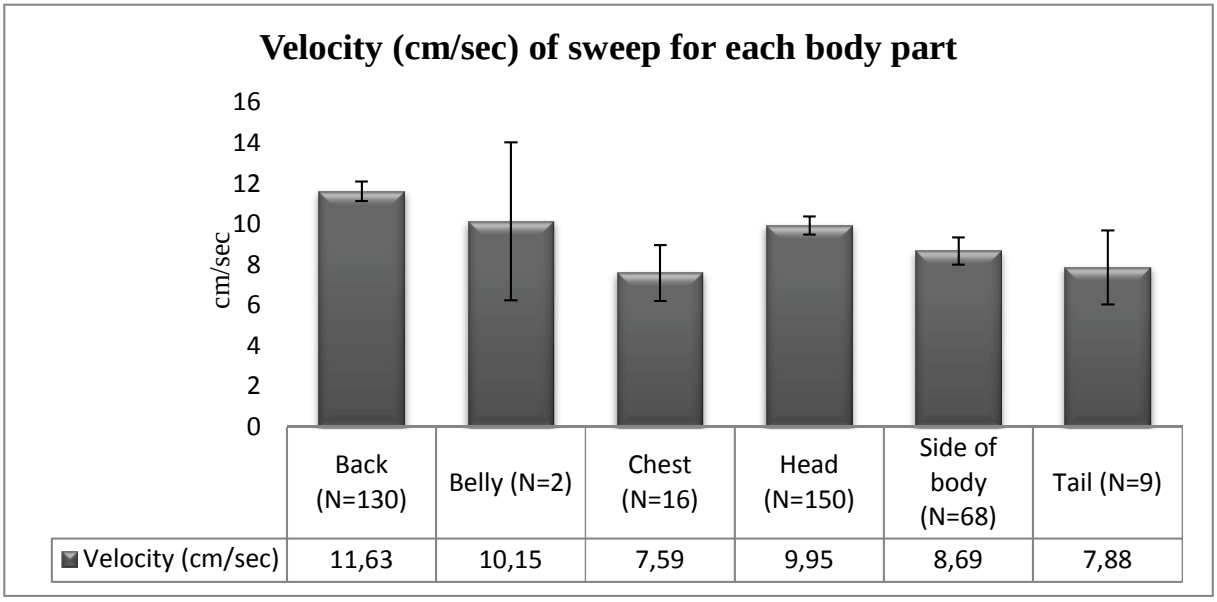


Figure S1. 1 *Velocity of real sweeping motion among free ranging monkeys. The bars indicate the standard errors.*

Conclusion and Discussion

In the present study, we analyzed the kinematics of the sweeping grooming movement among semi-free ranging monkeys in order to determine the velocity of the movement.

Human studies underlined that tactile simulation at the velocity of 1–10 cm/sec has been evaluated as pleasant and activated CT fibers (Morrison, 2012; Valbo *et al.*, 1995; Liljencrantz and Olausson, 2014; Billhult *et al.*, 2009). Here we reported the velocity of the sweeping on the back was 11.63 cm/sec, on the belly was 7.59 cm/sec, on the head was 9.95 cm/sec, on the side of body was 8.69 cm/sec and on the tail was 7.88 cm/sec. Any statistical difference among velocities were found. The mean value of velocity, independently from the body part, was 9.31 cm/sec, therefore inside the range of the optimal velocity to activate the CT fibers in humans (1-10 cm/sec). The data provide a first set of indirect evidence to Dunbar's hypothesis (Dunbar, 2010) about the correlation between activation of the CT fibers and the sweeping movement of grooming.

Even further studies will be necessary to confirm such correlation by means of directly measurements of fibers, the present study represents the first evidence that the velocity of the sweeping motion occurred during allogrooming among non-human primates is in the range of optimal velocity to activate CT fibers in human (1-10 cm/sec).

Heart rate and heart rate variability of male Rhesus monkey receiving human sweeping: Study 2

(Grandi LC, Roda' F, Ishida H. (2015). Physiological effect of sweeping grooming movements in macaque monkey: preliminary data. Journal of Primatology)

Aim of the study

The aims of the present study were to investigate for the first time in macaque monkey the velocity of the sweeping motion and the autonomic response during sweeping, in terms of heart rate and heart rate variability. For this purpose, we evaluated 1) if the velocity of the sweeping movement during conspecific allogrooming would be within the range of optimal velocities required to activate the CT fibers in human by means of a kinematic analysis; and 2) the heart rate and the heart rate variability responses when a monkey is receiving a sweeping caress from the experimenter at 4 different velocities (0.5-1 cm/sec, 5 cm/sec, 10 cm/sec and 20 cm/sec). The velocities of 5 and 10 cm/sec were chosen since they should be the optimal velocities for stimulation of the CT fibers, while the first and the fourth velocities should be the non-optimal ones (Löken *et al.*, 2009; Liljencrantz and Olausson, 2014) .The preliminary data here presents could be considered an important starting point in the studies related the CT system in non-human primates.

Material and Method

Subject of the EKG Experiment

We performed electrocardiogram (EKG) recordings of one experimental male Rhesus monkey (*Macaca mulatta*) aged 5 years, and weighing 5.6 Kg. 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, Bugugiate, Italy; approximately 180 cm height, 90 cm width, 120 cm depth) in an air-conditioned room maintained at a consistent temperature of 25–26 degrees Celsius. The monkey had access to toys, mirrors and swings in his own cage, while having visual, auditory and olfactory contact with other monkeys and being able to touch and be touched by neighboring ones. Any possible pain associated with surgeries was pharmacologically ameliorated, while the well-being and health conditions of the monkeys were constantly monitored by the institutional veterinary doctor of the University of Parma (Italy).

Recording Procedures

Before proceeding to record with the EKG, the monkey was habituated to sit in the primate chair within the laboratory, and to both interact and familiarize with the experimenter. At the end of the familiarization process and the training, a head fixation system was implanted. The surgery was performed under general anesthesia (ketamine hydrocloride, 5 mg/Kg, i.m. and medetomidine hydrocloride 0.1 mg/Kg i.m.), followed by post-surgical pain medication (Ishida *et al.*, 2013).

The EKG recording was conducted in the laboratory, and in the morning before feeding the monkeys. In the first experiment the EKG values were recorded in 3 different conditions, each for 2 consecutive minutes: Rest (as a baseline), Sweeping forearm (SA) and Sweeping back (SB). During Rest condition there was any physical contact between monkey and experimenter. For both the SA and SB conditions, the sweeping movement was performed at 4 different velocities: Very Low (VL), corresponding to 0.5-1 cm/sec; Slow (S) at 5 cm/sec; Fast (F) at 10 cm/sec; and finally Very Fast (VF), where the movement was 20 cm/sec. The experimenter was trained to perform the sweeping movements at these velocities. A one-minute non-recorded period was inserted between each condition in order to minimize any potentially overlapping effects. For each day, we recorded 1 Rest condition and one or more of each sweeping condition. The Rest baseline was always taken as the first condition, while the sweeping conditions were not recorded in the same order each day, but rather the order was randomly altered. In total, we acquired 23 trials for Rest, F_SA, S_SA, VL_SA, F_SB, VL_SB, and 10 trials for S_SB, VF_SA and VF_SB.

Data Collection and Analysis

The EKG activity was collected with surface electrodes (Medtronic®), and Spike2 was used to acquire the signal at the 1000 Hz sample rate. Each condition was recorded for 2 minutes, with the central 1 minute period analyzed. The heart rate RR interval values (time between 2 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 heart rate variability parameters. In respect to the frequency domain analysis of the heart rate variability, the power spectrum (of each 1 minute analyzed) 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 ($msec^2$), with the intervals of the power of the HF bands being in accordance with the literature (Champeroux *et al.*, 2013;

Uchiyama *et al.*, 2007). The power of the HF band is due to the activity of the parasympathetic nervous system, and therefore to the vagal tone activation (Berntson *et al.*, 1997; Eckberg, 1997).

In the time domain of the heart rate variability, we evaluated the square root of the mean of the squares of the successive differences (RMSSD, msec) between adjacent RRs, the measurement of short-term variation estimated the high frequency variations in heart rate, and therefore the activity of the parasympathetic nervous system (Malik, 1997).

In order to compare the heart rate and heart rate variability parameters among the conditions, statistical analyses were carried out using Statistica Software (StatSoft). The normality and equality of variance of the data (for each parameter in each condition) were verified with the Kolmogorov Smirnov and Levene tests, respectively. Since the normality was not verified, we employed non-parametric analysis.

The first analysis was performed in order to compare the heart rate and heart rate variability at Rest, and during each sweep stimulation. For this purpose, the Mann–Whitney U Test was performed test ($p < .05$). Then, the Kruskal-Wallis Test ($p < .05$) was performed in order to compare the effect of stimulation on the arm and on the back at the 4 aforementioned velocities, in terms of the heart rate and heart rate variability. The aim of this analysis was to investigate whether sweeping with different velocities would modulate the heart rate and heart rate variability differently.

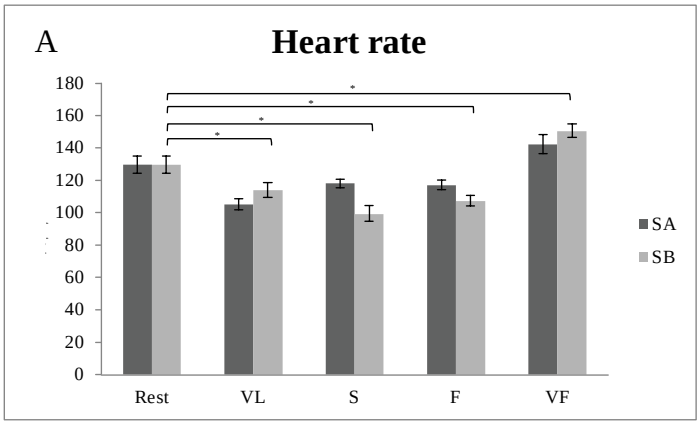
Result

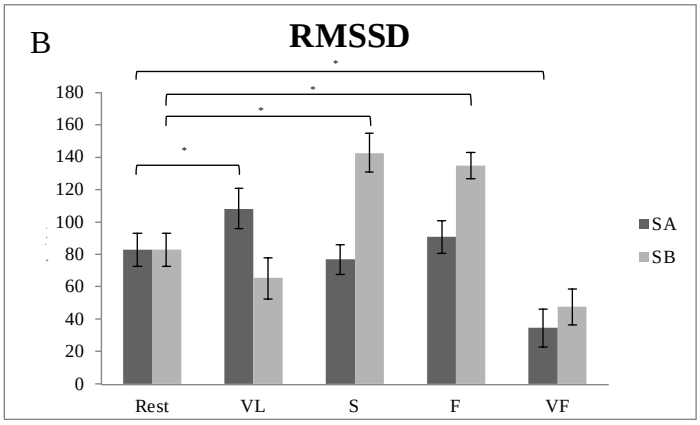
Heart rate and heart rate variability during rest condition (baseline) and sweeping back and arm

The first analysis was performed in order to investigate the modulation of the heart rate (HR) and each heart rate variability parameter (RMSSD and HF) during each of the 2 sweeping conditions (SA and SB) in the 4 velocities, in comparison to the baseline (Rest). The conditions of the SA, to be compared to the baseline, were: VL_SA, S_SA, F_SA and VF_SA. Precisely the same velocity comparison was carried out with the SB condition: VL_SB, S_SB, F_SB and VF_SB.

Sweeping back condition: The HR (mean of the 1 minute recording) during the baseline was statistically higher than during the sweeping back conditions at Very Low, Slow and Fast velocities ($p < .05$ for each comparison), and statistically lower than during sweeping at Very Fast velocity ($p < .05$; see grey bars of the Fig. S2.1A). The RMSSD and the HF during the baseline were statistically lower than during sweeping at both Fast and Slow velocities ($p < .05$ for each comparison), but did not differ from both Very Low and Very Fast velocities (grey bars of the Fig. S2.1B and Fig. S2.1C).

Sweeping arm condition: The HR (mean of the 1 minute recording) did not reveal statistically different modulation in any of the comparisons with Rest–Very Low velocity, Rest-Slow velocity, Rest–Fast velocity and Rest–Very Fast velocity (black bars of the Fig. S2.1A). The RMSSD during the baseline was statistically lower than during sweeping arm at Very Low velocity ($p < .05$), higher than during sweeping arm at Very Fast velocity ($p < .05$), but did not differ from both the Slow and the Fast velocities (black bars of the Fig. S2.1B). The HF during the baseline did not show statistically different modulation in any of the comparisons with Rest-Very Low velocity, Rest-Slow velocity, Rest-Fast velocity and Rest-Very Fast velocity (black bars of the Fig. S2.1C).





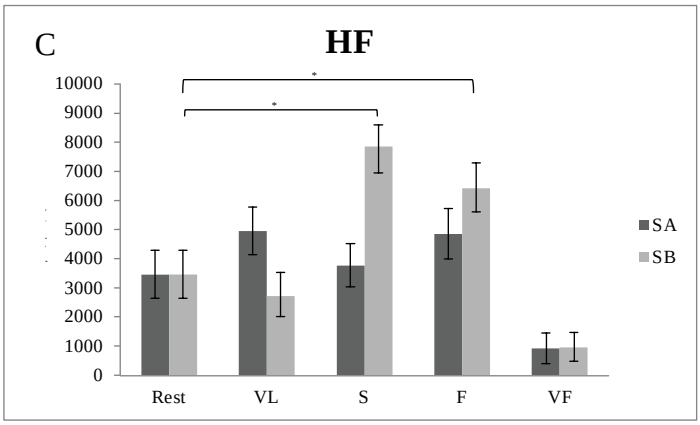


Figure S2. 1. *Comparison between baseline and sweeping stimulations.*

A) *The graphs represent the heart rate for Rest, the 4 sweeping arm conditions (black bars: VL_SA, S_ SA, F_ SA and VF_SA) and the 4 sweeping back conditions (grey columns: VL_SB, S_ SB, F_ SB and VF_SB).*

B) *The graphs represent the RMSSD for Rest, the 4 sweeping arm conditions (black bars: VL_SA, S_ SA, F_ SA and VF_SA) and the 4 sweeping back conditions (grey columns: VL_SB, S_ SB, F_ SB and VF_SB).*

C) *The graphs represent the HF for Rest, the 4 sweeping arm conditions (black columns: VL_SA, S_ SA, F_ SA and VF_SA) and the 4 sweeping back conditions (grey columns: VL_SB, S_ SB, F_ SB and VF_SB).*

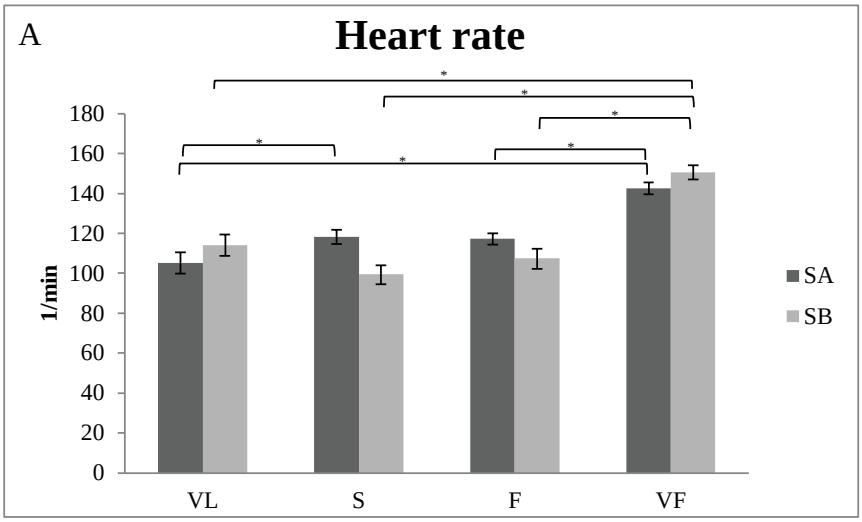
*In **A), B)** and **C)** the bars indicate the standard errors and the asterisks represent the statistically significant difference between the conditions ($p < .05$; Mann–Whitney U Test, for each parameter for each comparison). SA = Sweeping forearm; SB = Sweeping back; VL = Very Low velocity, corresponding to velocity 0.5-1 cm/sec; S = Slow velocity at 5 cm/sec; F = Fast velocity at 10 cm/sec; VF = Very Fast velocity, corresponding to velocity 20 cm/sec.*

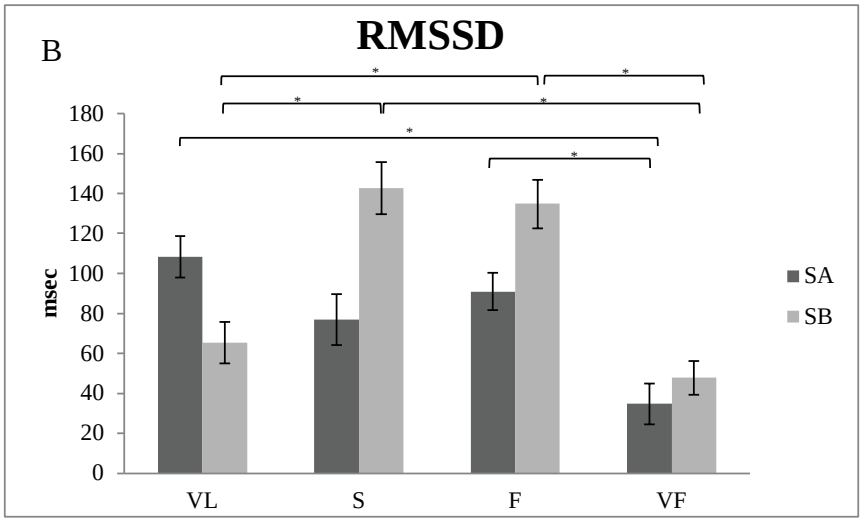
Heart rate and heart rate variability during sweeping back and arm

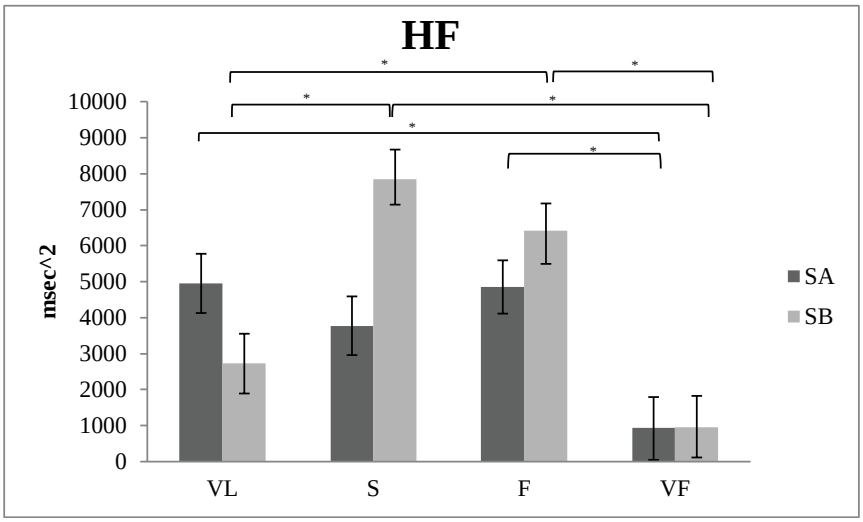
The second analysis was performed in order to evaluate whether the sweeping back and/or arm had a dissimilar effect on the heart rate and/or heart rate variability in relation to the velocities applied.

Sweeping back condition: The HR was statistically higher during sweeping back performed at Very Fast than at Fast, Slow and Very Low velocities ($p < .05$), although there was no statistically significant difference among the latter 3 conditions (grey bars of the Fig. S2.2A). The RMSSD and the HF were statistically higher during both Fast and Slow than during Very Fast and Very Low ($p < .05$), but there was no statistical difference between the first 2 (Fast and Slow) and the latter 2 (Very Fast and Very Low) conditions (grey bars of the Fig. S2.2B and S2.2C).

Sweeping arm condition: The HR during sweeping arm at Very Fast was statistically higher than during sweeping at Fast and Very Low velocities. Moreover the heart rate was higher during sweeping arm at Slow velocity than at Very Low one ($p < .05$ for each comparison; black bars of the Fig. S2.2A). The RMSSD and the HF were statistically higher during sweeping arm at Fast than Very Fast velocity, and during sweeping arm at Very Low than Very Fast velocity ($p < .05$ for each comparison), but there was no statistical difference in all the other SA-related comparisons (black bars of the Fig. S2.2B and S2.2C).







C

Figure S2. 2. *Comparison among sweeping stimulations.*

A) *The graphs represent the heart rate for the 4 sweeping arm conditions (black columns: VL_SA, S_ SA, F_ SA and VF_SA) and the 4 sweeping back conditions (grey columns: VL_SB, S_ SB, F_ SB and VF_SB).*

B) *The graphs represent the RMSSD for the 4 sweeping arm conditions (black columns: VL_SA, S_ SA, F_ SA and VF_SA) and the 4 sweeping back conditions (grey columns: VL_SB, S_ SB, F_ SB and VF_SB).*

C) *The graphs represent the HF for the 4 sweeping arm conditions (black columns: VL_SA, S_ SA, F_ SA and VF_SA) and the 4 sweeping back conditions (grey columns: VL_SB, S_ SB, F_ SB and VF_SB).*

*In **A), B)** and **C)** as convention of Figure 2 for errors, asterisks and abbreviations.*

Conclusion and Discussion

In the present study, we analyzed the heart rate and the heart rate variability of a monkey receiving sweeping from an experimenter on different body parts, in order to investigate the physiological effect of this particular tactile stimulation.

In order to investigate the cardio-physiological effect of the sweeping movement, we evaluated the heart rate and the heart rate variability of a male macaque monkey while receiving the sweeping movements from an experimenter on different body part (the arm and the back), at 4 different velocities. We demonstrated that the sweeping on the back determined a significant decrement of the heart rate and increment of the heart rate variability, if conducted at the 2 velocities of 5 and 10 cm/sec. However, the sweeping movement on the arm did not determine any such autonomic changes.

During allogrooming in semi-free ranging monkeys, they appear to show a preference for grooming on the back, while they usually ignore their arms for the target of allogrooming, although they perform self-grooming on their own arms. The preference for the back could be support by the physiological results of the present research, which demonstrated the positive effect of sweeping on the back as opposed to the arm.

A hypothetical reason of the difference in terms of modulation of heart rate and heart rate variability during the sweeping of the arm and back of the monkey, could be the differing densities of CT fibers in the 2 body parts. Even as so far there are any study in non-human primates, it was demonstrated in mice (Liu *et al.*, 2007) that these fibers are most densely distributed on the back, being sparsely present on the limbs, and completely absent from the paw skin. Moreover it was suggested (Walker and McGlone, 2014; Ackerley *et al.*, 2014) that the anatomical distribution of these nerve fibers determines people's emotional responses to touch depending on the interested body part.

Importantly, the present data suggest that the sweep done by experiments could be effective to create stronger bonds between experimental monkeys and experimenters, and to reduce the stress which animals could be exposed in various experimental conditions. Recently, Viktor and Annie Reinhardt (2008) reviewed the literature regarding the refinement of housing practices for single-house-cage experimental non-human primates. In the book they mentioned the positive contact with personnel, among many existing methods to reduce stress and increase welfare. They underlined that despite the broad consensus on the benefits of such physical contact, there is no published data to support this hypothesis. In agreement with this claim, our data first provide the evidence of the positive relaxing effect of tactile contact between a human and experimental monkey. The present

results were obtained from one male monkey, and this renders it difficult to interpret the results more broadly. Nevertheless this study implies a homology between human and non-human primate affective systems. In fact, human studies highlighted that the caress and moderate massages are utilized to improve the well-being not only for people suffering from depression, chronic pain, stress, neurological or psychological disease, and for cancer patients receiving chemo- and radio-therapy, but also to reduce the stress experienced by healthy people (Garner *et al.*, 2008; Billhult *et al.*, 2009; Diego and Field, 2009; Lindgren *et al.*, 2010). Further studies are required in order to investigate the CT fibers' density over the monkey's body and the link between the HRV and CT fibers' density and activation during sweeping. In conclusion the present study represents the first evidence that the human sweeping to the back of the monkey determined a decrement of HR and increment of HRV if conducted at velocities of 5 and 10 cm/second and that the physiological effect of human sweeping on the back of the monkey depended on the body part and of the velocity of the motion.

Considering the positive physiological effect of this kind of tactile stimulation, the results underlined that the sweeping back but not arm could be useful in order to improve the welfare of experimental monkeys.

Nose skin temperature, heart rate and heart rate variability of male Rhesus monkey receiving human sweeping:

Study 3

(Grandi LC, Heinzl E. (2015). Use of infrared thermography, heart rate and heart rate variability in studying effect of sweeps in Rhesus monkey. Special issue of ISAN2015: Autonomic Neuroscience. 192: 78. doi: 10.1016/j.autneu.2015.07.080).

Aim of the study

The aim of the present study was to investigate for the first time the effect of sweeping back in terms of nose skin temperature changes and to investigate the possible correlation with the HR and the HRV. We analyzed the nose skin temperature by means fIRT and the HR and the HRV by means of electrocardiograms recordings. The body part stimulated (back) and the sweeping velocities (1-3 cm/s Very Slow; 5-10 cm/s Medium; 16-20 cm/s Very Fast) were chosen based on the results of our previous study (Grandi *et al.*, 2015). In that study we underlined that the sweeping back of a male Rhesus monkey at velocity of 5 and 10 cm/sec determined the decrement of HR and increment of HRV. The sweeping with higher and lower velocities, as the sweeping arm, did not determined any positive physiological effects. Moreover the velocity of 1-10 cm/s is the optimal one to induce positive physiological reaction in humans (Löken *et al.*, 2009), by means of activation of CT fibers.

The functional infrared thermography (fIRT), measuring the energy radiating from the subject and translate it in temperature rate, is a useful non-invasive technique to study both positive and negative emotions in humans (Ioannou *et al.*, 2014). Nevertheless, even the growing importance of the use of infrared thermography to investigate the physiological effect of emotions on the body temperature changes, it has never been used to examine the physiological consequences of the affiliative touch, neither in human nor in non-human primate.

Despite the many human fIRT studies, the literature related the non-human primates in the field of thermography is very scarse. The first pioneer studies were conducted in 2005 and 2011 by Nakayama and colleagues and Kuraoka and colleagues, respectively, while the third and the last one was conducted by Ioannous and colleagues in 2015. In the first 2 experiments, the monkeys were exposed to negative stimuli and it was demonstrated the decrement of the nose temperature. In the last study, it was demonstrated the increment of the nose temperature of monkeys during a positive related situation, *e.g.* during playing. From those studies we may conclude that the nose temperature of monkeys increases when the animal is exposed to a positive situation and decreases if the situation assumes a negative value.

Material and Method

Subject

See please the paragraph **Subject of the *Study 2*.**

Recording Procedure

The measurements were performed in the morning before feeding monkey, inside the laboratory. The laboratory was a familiar environment for the monkey, since the same of the previous experiments. Before each session, the monkey was seated in the primate chair, bring to the laboratory and here 1) the head was fixed with the head holder and 2) the EKG electrodes were attached on the back of the monkey. The IRT camera was set in front of the monkey chair before bringing the monkey in the laboratory. The experiment started after 10 min from the attaching electrodes on the back, in order to allow the monkey to completely adapt to situation (Nakayama *et al.*, 2005).

Each session consisted of 2 phases of 2 minutes each: (1) pre-stimulation period, during which the monkey stayed quite without any physical auditory and visual contact with the experimenter; (2) stimulation period, during which the experimenter swept the back (about 5 cm) of the monkey. The stimulation period consisted of 1 of the 3 different swept: sweep at velocity of 1-3 cm/s, or 5-10 cm/s or 16-20 cm/s. Therefore we had 3 different sessions: rest-sweep at velocity of 1-3 cm/s, rest-sweep at velocity of 5-10 cm/s and rest-sweep at velocity of 16-20 cm/s. Between each session a period of 30 sec was inserted in order to minimize any potentially overlapping effect. The order of the sessions was randomly selected by experimenter. The number of sessions for each day was chosen day by day, in accordance to the monkey's behavior. In total we collected 12 trials for sweep at velocity of 5-10 cm/s and 9 trials of the sweep at velocity of 1-3 cm/s and 16-20 cm/s.

After each day of recording, the monkey was returned to own cage, feed and drank.

Data Collection

For EKG recordings, we considered the power of the high frequency (HF; 0.15–0.5 Hz) bands, expressed in absolute values (ms^2). The intervals of the power of the LF and HF bands were in accordance with the literature. In the time domain of the HRV, we evaluated the square root of the mean of the squares of the successive differences between adjacent RRs (RMSSD, ms), that is a measurement of short-term variation estimate high-frequency variations in HR, and therefore the

activity of parasympathetic nervous system. For detail about the Data Colletion, see please the paragraph **Data Collection of the *Study 2*.**

For the thermal recording, it was used a portable IRT camera (NEC Avio TVS500; Nippon Avionics Co., Ltd, Tokyo, Japan) with standard optic system, a resolution of 320x240 pixel, a emissivity of 0.97 , and an accuracy of 0,04° C. it was placed at 80 cm in front of the monkey's face. The nose skin temperature was recorded about every 20 s/ms. The imagines was analyzed with Grayess IRT Analyzer® PRO Version 6.0 Software. Every imagines was corrected for environmental and reflected temperature. A total of 875 images were exacted.

Statistical Analyses

For each condition (rest and the 3 sweeps) it was calculated the mean value of HR and each HRV parameters for the central 1 minute of the 2 recorded minutes. Concerning the IRT analysis, it was selected the best images within the 2 recorded minutes for each condition. Then it was calculated the mean temperature value of the nose according to the condition (rest, 3 sweeps). For both HR and HRV and thermal data, the Wilcoxon test was performed for thermal and cardiac data in order to compare each sweep condition (at 3 velocities), and the relative rest (pre stimulation) condition. This analysis was performed in order to evaluate the effect of sweeping at different velocity in comparison to the rest condition, where no physical contact happened. Then the Kruskal-Wallis Test was then performed in order to compare the 3 sweeping conditions at the 3 velocities. The aim of this analysis was to investigate if sweeping at different velocities would modulate the nose temperature and/or the HR and/or HRV differently. For the thermal data, we also performed the correlation analysis (Spearman's rank correlation), in order to ensure that the temperature of the nasal region was not dependent on the room temperature, for Rest and the 3 sweeping conditions (Nakayama *et al.*, 2005).

Result

The correlation analysis (Fig. S3.1) between each conditions (pre stimulation and each of the 3 stimulations) and the ambient temperature, underlined the absence of any relationship between ambient and monkey's nose temperature. Since the absence of correlation, it was possible to assume that any changes of the nose temperature was correlated to the relative condition.

The figure S3.2 shows 2 examples of thermal images of monkey, in which the nasal skin temperature in B) is higher than in C).

		Baseline	VerySlow	Medium	VeryFast
AmbientBaseline	Coef.Corr	***0,179***	*-0,024*	*0,52*	*0,087*
	Sign. (2-tails)	***0,344***	*0,94*	*0,152*	*0,825*
	N	***30***	*12*	*9*	*9*
AmbientVerySlow	Coef.Corr	*.a*	***.a***	*.a*	*.a*
	Sign. (2-tails)	*.a*	***.a***	*.a*	*.a*
	N	*12*	***12***	*9*	*9*
AmbientMedium	Coef.Corr	*0,183*	*-0,091*	***0,456***	*0,126*
	Sign. (2-tails)	*0,638*	*0,815*	***0,217***	*0,766*
	N	*9*	*9*	***9***	*8*
AmbientVeryFast	Coef.Corr	*-0,323*	*-0,581*	*0,481*	***-0,452***
	Sign. (2-tails)	*0,397*	*0,101*	*0,227*	***0,222***
	N	*9*	*9*	*8*	***9***

Figure S3 1. *Correlation map among the temperature of the ambient and each conditions (Spearman's rank correlation).In green the interest correlations. **Corr.Sign. p< .01 (2-tails). *Corr.Sign. p< .05 (2-tails).a One variable is constant. Correlation not possible.*

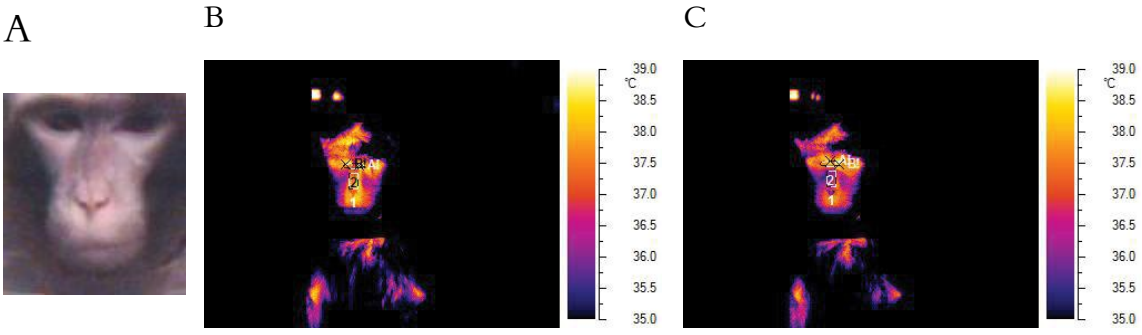


Figure S3 2. *An example of changes in the nasal skin temperature of the monkey. A) A photograph of the monkey. B) and C) Thermical images of Reshus Monkey, with remark points. The nasal skin temperature in B) is higher than in C).*

In the first analysis we verified that the 3 pre stimulation periods did not differ each others, in terms of nasal temperature, HR and HRV. The similarity among the 3 baselines is a pre requisite, since during the pre stimulation period of each sweeping condition, the monkey did not expected any stimulation and also did not know if and which of the 3 sweeps he will receive.

Once verified the absence of any differences among the 3 baselines, we investigated the effect of each sweep in relation to the relative baseline. The HR was statistically higher during pre stimulation than during stimulation, for each comparison (slow, medium and fast). RMSSD and HF were statistically higher during pre stimulation than during sweeping at fast and slow velocity but we did not find any difference between baseline and sweeping at medium velocity in terms of both RMSSD and HF (Fig. S3.3).

The nose temperature was statistically lower during baseline than during sweep, for each comparison pre stimulation- stimulation. (Fig. S3.4)

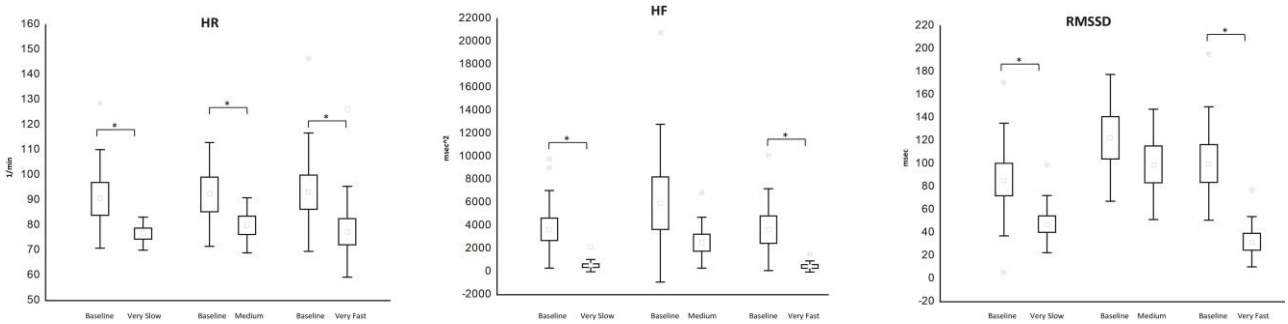


Figure S3 3. *The graphs represent the HR, the time domain parameter RMSSD and the frequency domain parameter HF for each pair Rest-Very Slow, Rest-Medium and Rest-Very Fast. The bars indicate the standard errors and the asterisks represent the statistically significant difference between the conditions ($p < .05$; Wilcoxon Test, for each parameter and each comparison).*

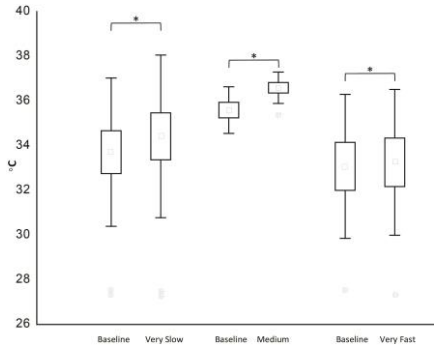


Figure S3 4 *The graphs represent the nasal skin temperature (^{o}C), for each pair Rest-Very Slow, Rest-Medium and Rest-Very Fast. The bars indicate the standard errors and the asterisks represent the statistically significant difference between the conditions ($p < .05$; Wilcoxon Test, for each comparison).*

The comparison among the 3 sweeping conditions, underlined that the HR did not differ, while the RMSSD and the HF were statistically higher during sweeping at medium velocity than during sweeping at slow and fast velocity. Moreover, the last 2 sweeping conditions did not differ each other (Fig. S3.5). The nose temperature was statistically higher during sweep at medium velocity than during sweep at fast velocity, but was not different from the temperature during sweep at slow velocity. We did not find difference between the temperature at slow and fast velocity (Fig. S3.6).

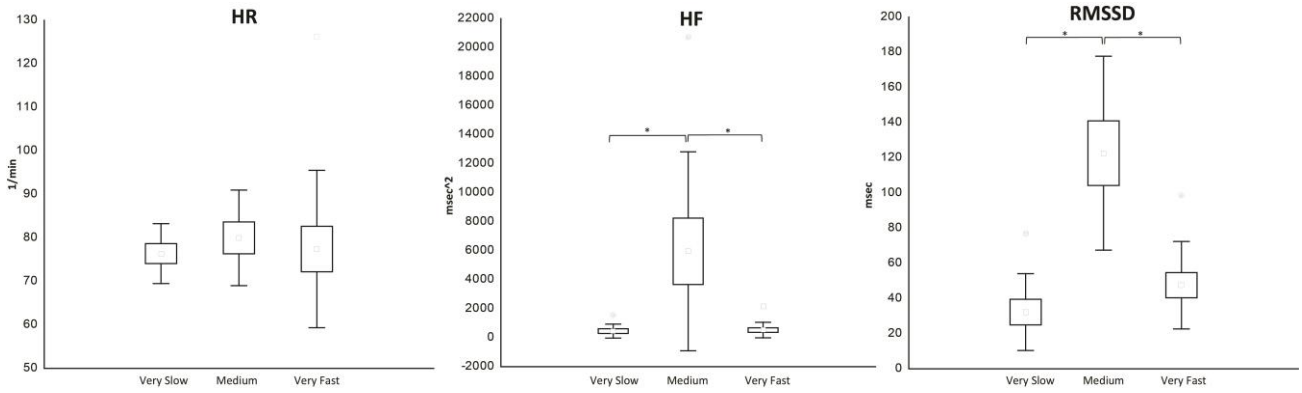


Figure S3 5. *The graphs represent the HR, the time domain parameter RMSSD and the frequency domain parameter HF for the comparison among the 3 velocities: Very Slow, Medium and Very Fast. The bars indicate the standard errors and the asterisks represent the statistically significant difference between the conditions (p < .05; Friedman Test, for each parameter).*

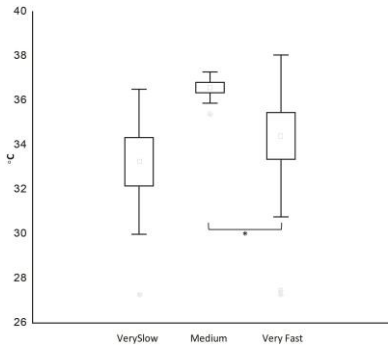


Figure S3 6. *The graphs represent nasal skin temperature (°C), for the comparison among the 3 velocities: Very Slow, Medium and Very Fast. The bars indicate the standard errors and the asterisks represent the statistically significant difference between the conditions (p < .05; Friedman Test).*

Conclusion and Discussion

The functional infrared thermography (fIRT) is nowadays an emergent and important non invasive technique to allow the investigation of the changes of the temperature at level of different body part in relation to different emotions. Even the many human studies, there are very few evidences related the non-human primates. Moreover there is no evidences related the effect of affiliative touch on the peripheral body temperature, neither in human nor in non-human primates, even the fIRT is a useful non invasive technique in order to detect the autonomic modulation in different emotional situations in humans (Ioannous *et al.*, 2014; Nummenmaa *et al.*, 2014). Nowadays there are just 3 investigation in the literature that analyzed the body temperature in relation to emotional stimuli in non-human primates. In those studies, it was demonstrated that the negative stimulations determined a decrement of the nose temperature (Nakayama *et al.*, 2005; Kuraoka *et al.*, 2011) while positive situations, such as playing, determined an increment of the nose temperature of the monkeys (Ioannous *et al.*, 2015).

In the present study we investigated for the first time the effect of a tactile stimulation, in terms of nose skin temperature changes, correlating it to the HR and HRV. Our results underlined that during the sensory sweeping stimulation the temperature of the nose of the monkey changes in comparison to the rest condition. Interestingly, the temperature changes depended on the velocity of the sweeping. In particular, the Medium velocity (5-10 cm/sec) determined a stronger increment of the temperature in comparison to the sweeping at low (< 1 cm/sec) or fast (20-25 cm/sec) velocities.

The analysis related the HR and the HRV underlined that the HR decreased during the sensory stimulation in comparison to the rest phase. The comparison among the 3 sweeping conditions revealed that the medium velocity had the best positive effects. In fact, during the sweeping at medium velocity the HR was lower and the HRV was higher than during sweeping at the slow and fast velocities.

Taken together the results underlined that both the cardiac and temperature related physiological parameters were affected by the sensory tactile stimulation in non-human primates in experimental situation. Interestingly the sweeping back performed by human at velocity of 5-10 cm/sec determined a decrement of HR, and an increment of HRV and of the temperature around the nose of the monkey. These results confirmed the previous data related the effect of sweeping back at velocity of 5 and 10 cm/sec, in terms of HR and HRV, and added the effect of the same stimulation in terms of body temperature.

Human studies demonstrated that the caress to the hairy side of the skin at a velocity of 1-10 cm/sec determined an increment of heart rate and increment of the heart rate variability of the

person that received it. Behavior analysis showed this touch is perceived as pleasant and coded as affiliative at central nervous system level. Our results related the HR and the HRV are in line with the human studies, since the sweeping at 5-10 cm/sec determined the decrement of HR and the increment of HRV.

Nevertheless there is no human and animal evidences about the changes of body temperature during a tactile stimulation. The non-human primate studies demonstrated that the aversive stimulations determined a decrement of the nose temperature, while a positive situation determined an increment of the nose temperature. Our results show that the tactile stimulation at 5-10 cm/se determined an increment of the nose temperature. Since the cardio physiological results revealed that the sweep at 5-10 cm/sec determined a positive autonomic modulation, by means of decrement of HR and increment of HRV, we could suppose that it is perceived as a positive stimulation by the monkey. Since positive situations determined an increment of the nose temperature, we could assume that the tactile stimulation at 5-10 cm/sec is a positive stimulation for the monkey.

Our results underlined for the first time that the temperature of the nose of the monkey changed during the tactile stimulation in comparison to the rest condition and that the touch performed with a velocity of 1-10 cm/sec determined stronger increment in comparison to the touch performed with higher velocity. Even if the present study represents a case report related the autonomic changes during a tactile stimulation, both in human and non-human primate, we believe it could be an important starting point in order to deeper investigate the effect of affiliative touch on the body temperature both in human (the caress) and in non-human primate (grooming).

Moreover, since we reported that the tactile stimulation at velocity of 1-10 cm/sec determined a decrement of heart rate, increment of heart rate variability and of temperature such stimulation could be useful in order to improve the welfare of experimental animal and to reduce their stress. In fact the results of the present study represent the first evidence of the positive relaxed effect of tactile contact between human and experimental single caged monkeys, as recently Viktor and Annie Reinhardt have hypothesized.

The effect of grooming carried out by humans on animals in relation to different body parts was studied by Normando and colleagues (Normando *et al.*, 2002) in respect to horses. In that study, the authors demonstrated that the HR of horses decreased during grooming in comparison to the rest period, and that there was a difference in terms of HR among groomed body parts; the HRV was not evaluated. Our results are partially in line with this study, since we detected the body part difference in term of HR. In addition we also underlined the body parts difference in terms of HRV. One hypothetical reason for the difference of HRV modulation during GA, GC and GM could be attributed to the different CT fibers distribution in the 3 body parts involved in this study. CT fibers

are low threshold mechanoreceptors of the hairy skin of various mammals, human and non-human primates (Bessou *et al.*, 1971; Douglas and Ritchies, 1957; Iggo and Kornhuber, 1977; Kumazawa, 1977; Light and Perl, 1979; McGlone *et al.*, 2007; Morrison, 2012; Sugiura *et al.*, 1986; Valbo *et al.*, 1995; Zotterman, 1939). The Social Touch Hypothesis identified them as a specific coding channel for gentle, dynamic touch occurring during close affiliative skin-to-skin interactions with conspecifics (Sugiura *et al.*, 1986; Valbo *et al.*, 1995; Olausson *et al.*, 2010; Roudaut *et al.*, 2012). Moreover, the polyvagal theory (Porges, 2007) proposed their impact on the heart rate modulation toward its decrement.

Since grooming can have an affiliative quality (Kapsalis *et al.*, 1996; Dunbar, 1991 and 2010), it could be considered as the equivalent of the social interpersonal skin-to-skin contact of humans. The studies (Lindgren *et al.*, 2012; Diego and Field, 2009; Field, 2014; Tsao, 2007; Billhult *et al.*, 2009; Belinda *et al.*, 2008; Schroeder *et al.*, 2014; Russell *et al.*, 2008) related to the consequences of affective interpersonal touch on the autonomous nervous system of humans underlined that this kind of tactile stimulation determines a decrement of HR and an increment of the HRV toward the vagal activation. Due to the positive effect on the autonomous nervous system, physical touch such as the caress and moderate massage are employed to reduce the stress of healthy people (Lindgren *et al.*, 2012; Diego and Field, 2009; Field, 2014), and might help convalescence for those suffering from depression, chronic pain (Tsao, 2007), stress (Billhult *et al.*, 2009; Belinda *et al.*, 2008), neurological disease (Schroeder *et al.*, 2014) and for cancer patients receiving chemo- and radio-therapy (Russell *et al.*, 2008).

Here, we report that the grooming carried out by familiar humans determines an increment of HRV parameters associated with the vagal activation. Importantly, we employed HRV analysis for the first time in order to evaluate the modulation of the autonomous system in psychologically related situations, such as grooming in Rhesus monkey. These results represent the first indirect evidence of the positive relaxing effect of the human-to-monkey grooming so we can assume that it has a positive autonomic effect (toward vagal modulation) comparable to the one evoked by the interpersonal skin-to-skin contact in humans.

Further investigations will be necessary to confirm that the vagal activation proposed by our results by means of HRV evaluation is also present during allogrooming among monkeys.

Due to these autonomic responses and the affiliative value of grooming for monkey, the data here presented could be useful in order to reduce the stress under which experimental animals could be for experimental conditions. Recently Viktor and Annie Reinhardt (2008) have hypothesized that the positive physical contact with personnel could be a method to increase the welfare of single house cage experimental non-human primates. Nevertheless, even the general consensus of it, there

is no published data to support this hypothesis. The results of the present study represent the first evidence of the positive relaxed effect of tactile contact between human and experimental single house cage monkeys, in term of autonomic response of monkeys.

Codification of sweeping in the secondary somatosensory cortex and insular cortex of male Rhesus monkey: Study 4

Aim of the study

Because of the key role of the posterior insula in the coding of affiliative interpersonal touch in humans (Olausson *et al.*, 2002 and 2010), and its hypothetical involvement as a neural substrate of the social behavior in non-human primates (Kling and Stein, 1976), here we investigate the modulation of the posterior disgranular and granular portions of insula of macaque monkey during a dynamic tactile stimulation, considered pleasant for non-human primate, *i.e.* the sweeping.

The monkey received a sweeping caress from the experimenter at two different velocities, 1-5 cm/sec and 5-13 cm/sec. The velocities of the two stimuli were chosen based on human studies and on the results of above presented *Study 1*,*2* and *3* since we reported that the real sweeping is performed with a mean velocity of 9.31 cm/sec, and that the sweeping performed with the velocity of 5 and 10 cm/sec determined the positive physiological effects on monkeys, in terms of decrement of HR and increment of body temperature and HRV, therefore the activation of vagal system. Moreover the caress at same velocity is perceived as pleasant, determines a positive modulation of autonomic system, modulates the activity of insular cortex and activates the CT fibers in humans (Löken *et al.*, 2009; Olausson *et al.*, 2002 and 2008 a,b,c). On the contrary, the sweep at velocity lower than 5 cm/sec does not have any positive physiological effect. Human studies underlined that the optimal velocity of pleasant dynamic touch that determined the activation of CT fibers, therefore positive autonomic effects and the modulation of the insular cortex, is in the range of 1-10 cm/sec (Löken *et al.*, 2009; Olausson *et al.*, 2002 and 2008 a,b,c).Taken together these evidence, we selected the velocity of 1-5 cm/sec since non optimal to induce positive autonomic effect in monkeys and the speed of 5-13 cm/sec since it includes the real sweep speed and it seems the optimal velocity to induce positive autonomic effect in monkeys.

In the present study we did not tested neurons with speed of sweeping higher that 15 cm/sec and lower than 1 cm/sec since these velocities of stimulation were uncomfortable for the monkey, as we already notice for the experiment of the *Study 2* and *Study 3* here presented, and as demonstrated from the increment of HR and decrement of HRV (*Study 2*). Moreover, the monkey started to struggle and rejected the stimulation after a while, and this would prevent the performing of the present study. We therefore decided to not test the neurons with those velocities.

Although human studies highlighted the importance of posterior insular cortex but not of sensory areas, we cannot exclude a priori the involvement of the secondary somatosensory cortex (SII) in the coding of the sweeping. Therefore we decided to investigate the modulation of both areas.

Moreover, we investigated the presence of similar neurons also from primary somatosensory cortex, in order to compare the neuronal modulation in the convexity and deep brain areas during sweeping. It is well known from literature that the primary sensory cortex receives peripheral tactile information from the large myelinated fibers involved in the codification of the discriminative aspect of the sense of touch. On the contrary, those areas do not receive information from slow unmyelinated fibers, which have role in the codification of affective meaning of the peripheral tactile stimulation.

Our hypothesis was that the insular cortices and SII were modulated during sweeping performed with velocity of 5-13 cm/sec but not during sweeping performed with lower velocities, while the primary sensory cortex should not discriminate between the two ranges of velocities. According to our hypothesis the investigated areas have a role in the coding of the velocity of a dynamic passive touch characteristic of affiliative pleasant behavior among Rhesus monkeys.

Material and Methods

Subject

The experiment was performed on one 5-year-old male Macaca mulatta (5.5 kg). Since the monkey was used as subjects of another previous electrophysiological experiment, he was already habituated to sit on a primate chair, to interact with the experimenters and to perform a fixation task, as described below (Fig. S4.1). Moreover also the head fixation system (Crist Instruments) and a circular (2 cm of diameter) stainless steel chamber (Narishige, Japan) on the left hemisphere were implanted under general anesthesia (ketamine hydrochloride, 5 mg/kg i.m. and medetomidine hydrochloride, 0.1 mg/kg i.m.), followed by post surgical pain medications. The here presented study was performed recording firstly on the left hemisphere and, once finished the recording sessions in that hemisphere, it was implanted the circular (2 cm of diameter) stainless steel chamber (Narishige, Japan) on the right hemisphere. The correct position of both chamber to reach the interested deep brain area to record it was establish based on fMRI of that monkey's brain. All surgical procedures were the same as previously described (Ishida *et al*. 2013). 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 monkeys were kept in individual primate cages consisting of full metallic grid (Tecniplast S.p.A. Buguggiate. Italy). Each cage was of 180 cm height, 90 cm width, and 120 cm depth. In the middle of the height of each cage, it was possible to insert one or two panels for the monkey to sit on. The litter was located immediately below the cage. In the bottom part of the cage, two containers were located, one for water and one for food, which were filled by the experimenter. The monkeys were provided with food and water once a day, usually in the morning. Inside the cage, each monkey had access to toys, mirrors, and swings. Moreover monkeys had visual, auditory, and olfactory contact to each other and were able to touch and groom with the neighboring monkeys. All cages were in an air-conditioned room maintained at a consistent temperature of 25–26°C. The well-being and health conditions of the monkeys were constantly monitored by the institutional veterinary doctor of the University of Parma (Italy).

Apparatus and behavioral paradigm

During the recording sessions, monkey had to perform a fixation task meanwhile receiving the sensory stimulation by experimenter. During the total length of the task, the monkey had to maintain the hand contralateral to recording hemisphere on a metal cylinder (diameter 3 cm, height 2.5 cm). fixed to the plane close to the monkey's body and the ipsilateral one laid on the plane. In front of the monkey at 80 cm of distance it was located a light-emitting diode (LED) that monkey had to fixate for all the task's length. The task (Fig. S4.1) consisted of:

1. Green_On – Green_Off: the green LED turned on for 0.5-2 sec.
2. Red_On: the green LED switched to red and the experimenter moved the hand toward the monkey's body part to be stimulated.
3. Trg2_On –Trg2_Off: experimenter touched the monkey (Trg2_On). The red LED switched off and the green LED turned on until the sensory stimulation finished (Trg2_Off).
4. Once the sweep finished, the green LED switched off and after 0.8-1 sec the monkey received a drops of juice as reward. After 1.5-2 sec, the next trial started. From the end of stimulation to the start of the next trial we did not requested monkey any fixation, but just to maintain the hands position.

The task consisted of 2 passive dynamic somatosensory stimulations (sweeps), randomly applied of the receptive field of each neuron: 1) Sweep with a speed of 1-5 cm/sec. We defined this condition as *Slow Sweep*; and 2) Sweep with a speed of 5-13 cm/sec. We defined this condition as *Fast Sweep*. The stimulation was dynamic since it was a kind of caress (chosen to imitate the sweeping performed by monkeys), performed by experimenter (by hand) at 2 different velocities.

The stimulations were manually applied by experimenter, who was trained in order to applied the stimulation with the two ranges of velocity and with the same pressure independently from the speed. The exact applied velocity was offline calculated and confirmed.

The body part and the direction of stimulation chosen to test each neuron were the best that elicited the activity of neuron, identified during clinical tests. The stimulation covered all length of the receptive field/fields (RF/RFs). The neurons that showed clear passive RF in more than one body part were tested in all identified RF but for the statistical analysis it was considered just the body part were the at least one of the two sweeps elicited the best neuronal discharge.

Due to the experimental design, we controlled the task in order to assure that the neuronal modulation was determined by just the passive sweep stimulation. In fact:

- The task was performed in a dark room and we requested monkey to maintain the fixation from the first green LED switched on to the finish of the sweeping stimulation, the monkey cannot see the action of experimenter. In this manner we avoid visual feedback and its possible influence on the neuronal activity. If monkey broke the fixation the trial was aborted and monkey did not receive any reward.
- We requested monkey to maintain the hands position, in order to avoid any influence determined by eventually active motor properties of neurons.
- The 2 sweeps (Slow Sweep and Fast Sweep) were randomly performed, so the monkey didn't know the type of sweep he will receive. avoiding the expectation of one or other stimulation.
- Once the red LED switched on, the experimenter decided trial by trail when started to move toward and touched the monkey's body. Monkey knew that once red LED switched on, after a while, but not exactly when he will receive a sweep (Slow or Fast).
- Once the sweep finished the monkey received reward. but he did not know exactly when. from 0.8 and 1 sec. In this manner we avoid the expectation of reward and possible modulation of neurons during this period. since in literature it was described the presence of reward related neurons in the anterior part of the Insular cortex (Mizuhiki *et al.*, 2012). but we cannot excluded the presence of such neurons in other portions of insula.

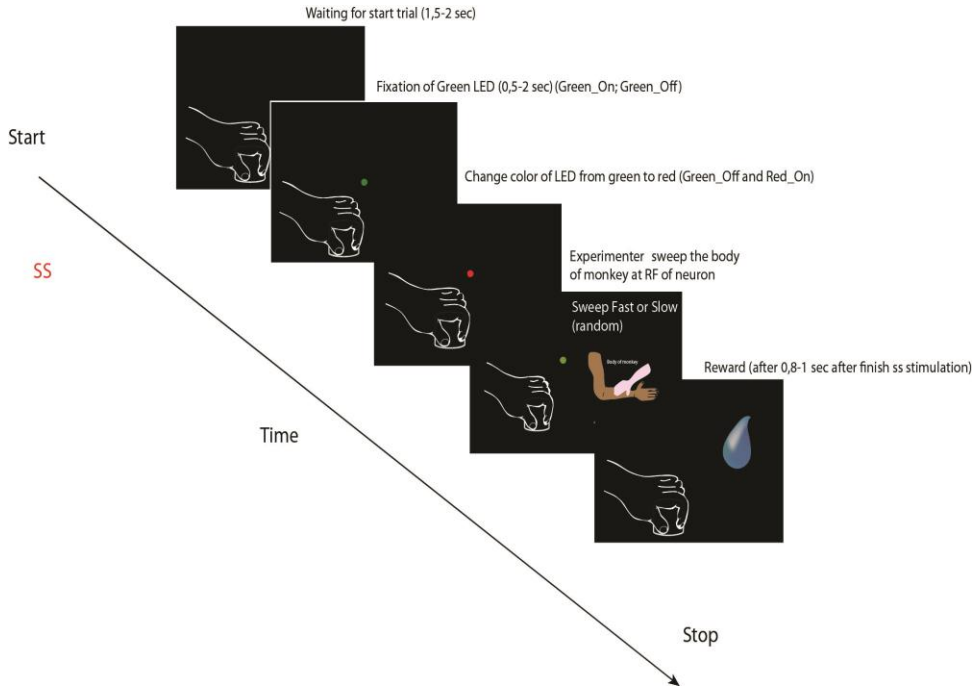


Figure S4. 1. *Somatosensory task: Sweep Slow and Sweep Fast. 1) Green_On – Green_Off: the green Light Emitting Diode (LED) turned on and the monkey had to fixate it for 0,5-2 sec and to maintain the hand contralateral to recording hemisphere on the home-key. 2) Red_On: the green LED switched to red and the experimenter moved the hand toward the monkey's body part to be stimulated. The monkey had to fixate the red LED and to maintain the hand contralateral to recording hemisphere on the home-key. 3) Red_Off/Green_On/Trg2_On – Green_Off/Trg2_Off: when the experimenter touched the monkey (Trg2_On), the red LED switched off and the green LED turned on until the sensory stimulation finished (Trg2_Off). The monkey had to fixate the green LED and to maintain the hand contralateral to recording hemisphere on the home-key. 4) Once the sweep finished, the green LED switched off and after 0,8-1 sec the monkey received a drops of juice as reward. We did not requested monkey any fixation, but just to maintain the hand contralateral to recording hemisphere on the home-key. 5) After 1,5-2 sec, the next trial started.*

Recording techniques

We recorded from the secondary somatosensory cortex (SII) and from disgranular and granular insular cortex (dIg and Ig. respectively), in both left and right hemispheres. For each penetration, the correct depth was estimated on the basis of the properties in different regions above, from first neuronal activity detection in the convexity. The position of the chambers were decided based on fMRI of the monkey in order to better reach the maximal possible length of SII. dIg and Ig. The figure S4.2 shows the position of the right chamber.

In the right hemisphere the extracellular single unit recordings were carried out using varnish-insulated tungsten microelectrodes (length 100 mm, impedance 1.5-3.0 MΩ at 1 kHz; FHC, USA), and we clinically tested neurons in the recording site, each 300 μm.

In the left hemisphere, the neuronal recordings were performed by means of 16 channel V probe. Both the V-probe and the tungsten microelectrodes were inserted vertically (90°) into the cortex through the intact dura matter using a hydraulic microdriver manipulator (Narishige. Japan). Each isolated single neurons in each of the 16 channels was firstly clinically tested. Then, after the first recording session, we moved down the V probe in the case the probe did not covered the entire interested area, in order to perform a second recording session. In the second recording session we considered just the channels where we discriminated new single neurons and not already recorded neurons in the first session.

In both left and right hemisphere, once reached the white matter at the end of recording sessions, we moved down the V probe/tungsten electrode until next cortex, in order to physiological confirmed the recording site. The neuronal activity was recorded by a Plexon system, then both the analogic and digital signal acquired on-line were sent to a PC and stored for a subsequent analysis. The off-line analysis was carried out using a specific Offline Sorter software (OmniPlex^{TM}).

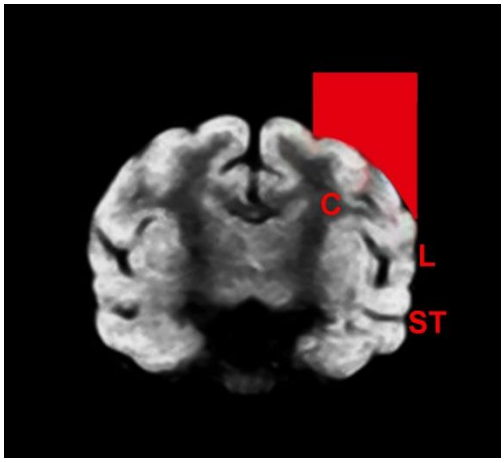


Figure S4. 2. *Coronal section of monkey's right hemisphere related the center of chamber. In red the chamber. Abbreviations: S = central sulcus. L = lateral fissure. ST = temporal sulcus***.**

Recording of behavioral events and definition of epochs of interest

The starting and the switching off of each LED during the task were monitor by a LabView-based software. Distinct contact-sensitive devices (Crist Instruments) were used to detect the maintenance of the hands position and when the experimenter begin and finish to touch the monkey's body. These devices provided a transistor– transistor logic signal (TTL), which was used by to monitor the monkey performance. Eye position was monitored by an eye tracking camera (ISCAN Camera, ETL-200). The eye position signals, together with the TTL events generated during task execution, were sent to the LabView-based software to monitor the progress of the task. Based on TTL and eye position signals. the software enabled us to automatically interrupt the trial if the monkey broke fixation and/or the hands positions. In these cases, no reward was delivered. The monkey always received the same amount of juice as a reward after correct performance of each trial. Based on the digital signals related to the LED and the two events determined by the contact between the hand of the experimenter and the body of the monkey, we defined two different epochs (Fig. S4.3) of interest for statistical analysis of neuronal responses: Baseline: from 500 ms to 900 ms after the Green_On (total of 400 ms); and 2) Stimulation: from Trg2_On to Trg2_Off.

We selected the time interval from 500 ms to 900 ms after the Green_On (total of 400 ms) as baseline since this interval was situated after the start of the trial and before the monkey received the sensory stimulation. In this interval, monkey did not see the hand of experimenter since had to fixate the LED and did not know when and which kind of sweep he will receive. The time interval selected for the stimulation is the exact interval of the duration of sensory stimulation for each condition and each trial.

Since the stimulation was manually performed by experimenter, without a strictly online control of the applied speed, before to perform any analysis, we verified that the speed of each trial in each of the 2 conditions was performed with the speed in the desired range, *e.g* 1-5 cm/sec and 5-15 cm/sec for *Slow* and *Fast Sweep*, respectively. In order to calculate the speed the cm for each tested body parts were divided for the exact time of the stimulation. The time of stimulation for calculated for each trial extracting the time of Trg2_On and of the Trg2_Off.

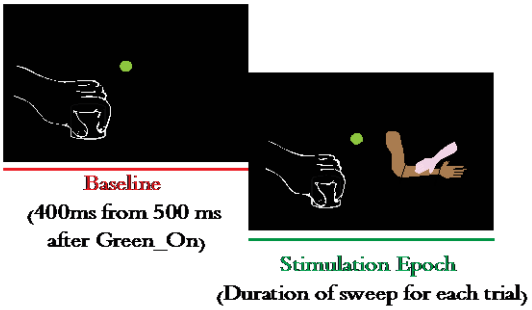


Figure S4. 3. The 2 epochs for statistical analysis.

Data analyses and classification of the recorded neurons

Single units were online isolated and the off-line analysis was carried out using a specific Offline Sorter software (OmniPlex^{TM}).

Definition of task related neurons

Neurons were defined as "task-related" if they significantly varied their discharge during at Stimulation epoch in at least one of the two conditions in comparison to the Baseline. The normality and equality of variance of the data (for each parameter in each condition) were verified with the Kolmogorov Smirnov and Levene tests, respectively. In order to identified the task related neurons we performed repeated measures 2 X 2 ANOVA (factors: Conditions and Epochs) followed by Bonferroni *post hoc* test in case of significant epochs and/or interaction conditions X epochs effects ($p < .05$). The post-hoc was execute in order to investigate possible differences 1) between epochs in each condition; 2) between conditions in each epochs and 3) between conditions independently to epochs.

The neurons showing the epochs effect but not statistical differences between the stimulation epochs of the conditions were classified as *Speed Unselective* neurons. The neurons showing the statistical different between the stimulation epochs of the 2 conditions were instead defined as *Speed Selective* neurons. Among the second category, we identified the *Fast Speed Selective* if they showed the stimulation epoch of Fast Sweep statistical higher that the one of the Slow Sweep. Viceversa for the and the *Slow Speed Selective* neurons.

The raster and histograms of single unit data show the firing rate (spike/sec) normalized for the maximal value between the two conditions in each single unit. The alignment is the Trg2_On, therefore when the experimenter started the stimulation to the monkey's body.

Correlation analyses

In order to investigate if the neuronal discharge of *Speed Selective* neurons correlated with the speed of applied speed, we performed the Correlation analysis. As control we performed the same analysis also for the *Speed Unselective* neurons. Since both categories showed a linear correlation, even in different percentage, we performed Mann-Whitney U-Test to compare the distribution of R-square. The Chi-square test was performed in order to assess the probability that observed value was near the real measurements.

Population analyses

Population analyses were performed on three categories of neurons, classified on the basis of the results of the above described analysis, and taking into account single-neuron responses calculated as normalized activity (spike/sec) in 20 ms bins, in the two tasks (Sweep Fast and Sweep Slow). The normalized activity for each neuron was calculated as follow: the mean activity was calculated every 20 ms bins in all the recorded trials of all the experimental conditions to be compared. Then, the absolute highest activity value was taken to divide the value of each single bin in all conditions (normalized mean activity, ranging from 0 to 1).

The three categories were *Speed Selective* , *Speed Unselective* presenting the correlation between firing rate and speed of stimulation and *Speed Unselective* without the correlation between firing rate and speed of stimulation. The histograms showed the moving average of 60 ms of the normalized activity, for each of the two trials. The alignment is the moment when the experimenter touch the body of the monkey.

In order to investigate the bins where the two conditions significantly differed each others, in terms of population, we performed 2-pairs T-Test ($p < .05$) for each of the three population. It was taking into account each 20 ms (1 bin) from the Trg2_On, therefore when the experimenter started the stimulation to the monkey's body, until 2 sec. We selected 2 sec as maximal time to analysis population data since the stimulation (both Sweep Fast and Sweep Slow) were finished within 2 sec. The minimum number of bins that we selected for significance was 3. For the population analysis we did not considered the Slow selective neuron in the population of Speed Selective neurons.

All statistical analyses were carried out using Statistica Software (StatSoft).

Single unit recording in primary somatosensory cortex: control experiment

For each penetration in both left and right hemisphere, we recorded also from primary somatosensory cortex (SI), before to record in SII and Insular cortex, tested each isolated neuron with same task using for the recording in the deep brain areas. The recording techniques, apparatus and behavioral paradigm, the data analyses and the definition of task related neurons were the same adopted for recorded neurons in SII and insular cortex.

Anatomical definition of recording sites

In order to confirm the recording sites, at the end of recording sessions and ten days before to sacrifice the monkey, electrolytic lesions (10 µA cathodic pulses, duration 10 s) were performed in both the hemispheres (Bonini *et al.*, 2011). In detail, for each hemisphere at known coordinates of the external borders of the recorded regions were selected and in each of them four lesions were made at different depth. Then, the monkey was deeply anesthetized with an overdose of sodium thiopental and perfused consecutively with saline, 3.5–4 % paraformaldehyde, and 5 % glycerol, prepared in 0.1 M phosphate buffer and pH 7.4, through the left cardiac ventricle. Each brain was then blocked coronally on a stereotaxic apparatus, removed from the skull, photographed, and placed in 10 % buffered glycerol for 3 days and 20 % buffered glycerol for 4 days (Gerbella *et al.*, 2014). Finally, it was cut frozen into coronal sections of 60-µm thickness and in order to identify the electrolytic lesions, we performed the Nissl staining (0.1% thionin in 0.1 M acetate buffer, pH 3.7).

The figure S4.4 shows a section of the right hemisphere with two electrolytic lesions (on the left) and the relative entire section (on the right). The section is related to the most posterior and medial electrolytic lesions, therefore the medial and posterior border of the recording sites. The asterisks in red represent the lesions. Moreover, the section presents also two tracks likely related to two different penetrations of the recording electrodes. The anatomical study confirmed that the recording sites are located in the physiological defined SII, dIg, and Ig.

Further deeply investigations and comparison of the physiological data with anatomical analysis could be useful to discriminate which neurons were recorded in dIg and Ig and which in SII. However, since a clear identification of the physiological properties defining the border between SII and Insular cortex is particularly difficult, the here presented results are related to both SII and insular cortex.

Results

We recorded a total of 223 task related neurons somatosensory neurons from both the right and the left secondary somatosensory cortex (SII), disgranular (dIg) and granular insular cortex (Ig) of one male Rhesus monkey (*Macaca mulatta*). In particular, we recorded 107 neurons in the right hemisphere and 116 neurons in the left hemisphere. Since the recording sites are deep brain areas, the correct depth of each penetration was estimated on the basis of the physiological properties of areas above the region of interest. Once reached the interested region, each neuron was clinically tested and if it presented one or more clear passive receptive fields (RFs) it was recorded with the tasks, *Sweep Slow* and *Sweep Fast*, both dynamic sensory stimulation. Moreover, the anatomical study confirmed the borders of the recording sites in both hemispheres (Fig. S4.4).

The task related neurons were defined based of the first analysis (2 x 2 ANOVA, please see Material and Method section) if they showed significant the main effect of the factor Epoch and/or interaction between factors Conditions and Epochs. Since the neurons were tested by two passive dynamic sensory stimulation, the task related neurons were considered as sensory neurons responsive for the passive dynamic stimulation at a specific receptive field/fields (RFs), but not static tactile stimulation.

The identified RFs where the sweeping was applied (Table S4.1), were the mouth (N=71; 31.84%), the hairy side of the hand (N=60; 26.91%), the face (N=21; 9.42%), the arm (48; 21.52%), the upper body (front side, *i.e.*. the chest and the belly; N=3; 1.35%), the leg (N=15; 6.73%) and the back (N=5; 2.24%). All neurons had bilateral RFs but the stimulation was applied on the contralateral side.

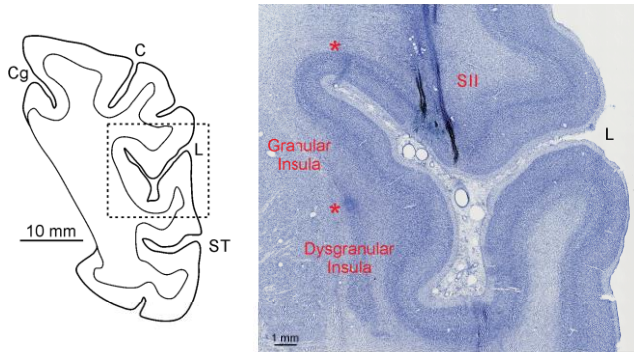


Figure S4. 4. *On the right: a part of a Nissl section of the right hemisphere. In this section it is possible to identify two electrolytic lesions (asterisk). These lesions were performed in the most posterior and medial side of the recording site. On the section, also two tracks of recording electrode through the SII. On the left, the reconstruction of the entire section and the dash square represents the relative part of section illustrated on the right. Abbreviation: L= lateral fissure; SII= secondary somatosensory cortex; ST = temporal sulcus; C = central sulcus.*

Speed selectivity of task related neurons

The task related neurons were divided in two categories, the *Speed Selective* and the *Speed Unselective neurons.* The *Speed Selective* neurons showed significant main effect of the factor Epochs and interaction between Conditions and Epochs factors. Therefore, they were modulated in one or both the two sweeping stimulation and the neuronal activity during one stimulation was significantly higher than the neuronal modulation during the other one. The *Speed Unselective* neurons showed significant main effect for just the factors Epochs. Therefore, they were modulated during both Sweep Slow and Sweep Fast and they did not show any statistical difference between the two.

Among the 223 task related neurons, 38 (17.04%) were *Speed Selective* and all the 38 *Speed Selective* neurons were excitatory neurons. The Table S4.2 shows the identified RFs of the *Speed Selective* neurons.

Thirty-seven of 38 *Speed Selective* neurons were *Fast Selective* neurons. Among the *Fast Selective* neurons, 14 neurons were activated during both conditions, but the neuronal discharge during the stimulation epoch of Sweep Fast was higher than the firing rate during the stimulation epoch of the Sweep Slow. Twenty-three out of 37 *Fast selective* neurons were activated just during the Sweep Fast condition, but not the Sweep Slow. The figure S4.5 shows an example of *Speed Selective* neurons activated just during Sweep Fast (*Fast selective*), while the figure S4.6 shows example of *Speed Selective* neurons activated during both Sweep Fast and Sweep Slow but the modulation during Sweep Fast was higher than during Sweep Slow (*Fast selective*).

Finally, just 1 of 38 was a *Slow Selective* neuron since it was activated just during the Sweep Slow but not during the Sweep Fast. The figure S4.7 shows the *Slow Selective* neuron.

RF	***Task related neurons***	
Mouth	70	(31,53%)
Hand	60	(27,03%)
Face	21	(9,46%)
Arm	48	(21,62%)
Upper body	3	(1,35%)
Leg	15	(6,76%)
Back	5	(2,25%)
Total	223	(100%)

Table S4. 1. *Identified Receptive field of the 223 recorded task related neurons.*

RF	*Speed selective*	
Mouth	11	(28,95%)
Hand	13	(34,21%)
Face	4	(10,53%)
Arm	5	(13,16%)
Upper body	3	(7,89%)
Back	2	(5,26%)
Total	38	(100%)

Table S4. 2. *Identified Receptive field of the 38 Speed selective neurons.*

RF	*Speed Unselective*	
Mouth	59	(32,07%)
Hand	47	(25,54%)
Face	17	(9,24%)
Arm	43	(23,37%)
Leg	15	(8,15%)
Back	3	(1,63%)
Total	184	(100%)

Table S4. 3. *Identified Receptive field of the 184 Speed unselective neurons.*

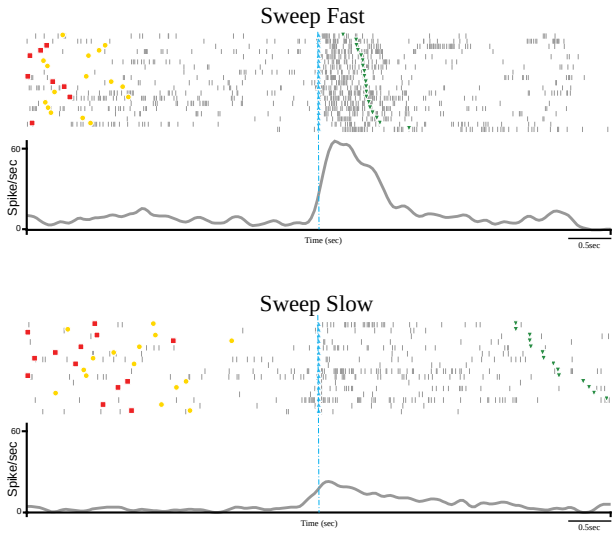


Figure S4. 5. *Example of Speed Selective neuron (Fast selective). The neuron was modulated just during Sweep fast. The neuron was recorded in the right hemisphere and the RF was located on the hairy side of bilateral hand. The stimulation was performed on the contralateral side, from the distal (fingers) to proximal part (wrist). The histograms are alignment at the moment when the experimenter touched the fingers of the monkey, indicated by the light blue triangle. The end of the stimulation is indicated by the green triangles. The time between red dots and yellow circles indicates the baseline, while the time between light blue and green triangles indicates the stimulation epoch. The histograms are normalized for the maximal spike/sec value between the two conditions.*

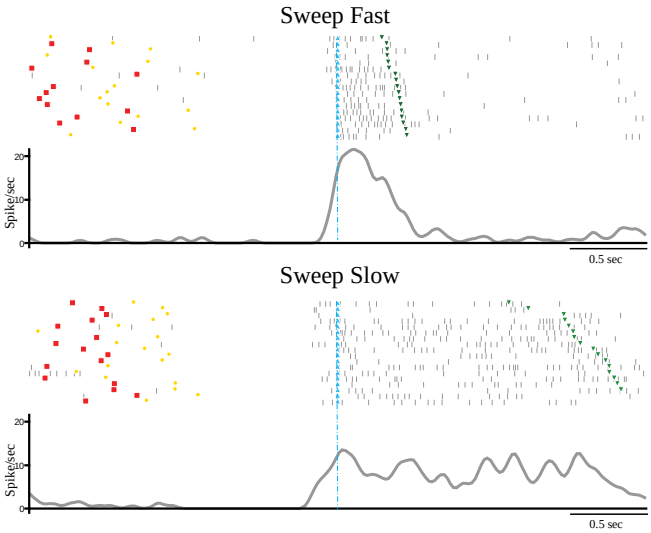


Figure S4. 6. *Example of Speed Selective neuron (Fast selective). The neuron was modulated during both sweeping but the activity during Sweep fast was higher than during Sweep slow. The neuron was recorded in the right hemisphere and the RF was located on the hairy side of bilateral hand. The stimulation was performed on the contralateral side, from the distal (fingers) to proximal part (wrist). The histograms are alignment at the moment when the experimenter touched the fingers of the monkey, indicated by the light blue triangle. The end of the stimulation is indicated by the green triangles. The time between red dots and yellow circles indicates the baseline, while the time between light blue and green triangles indicates the stimulation epoch. The histograms are normalized for the maximal spike/sec value between the two conditions.*

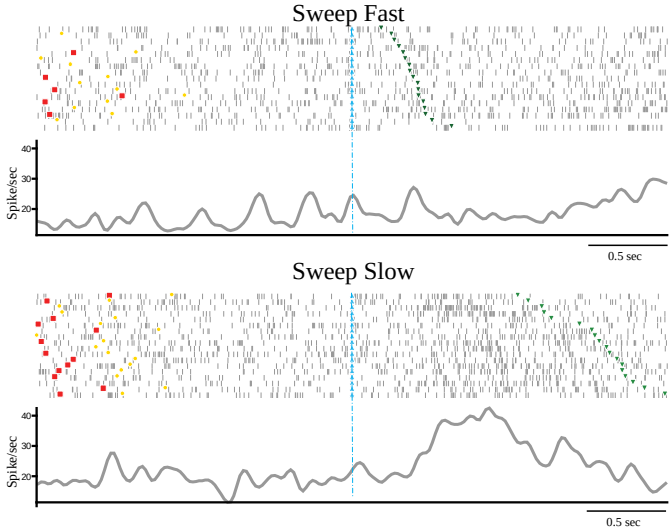


Figure S4. 7. *Slow selective neuron, activated just during the Sweep slow. The neuron was recorded in the left hemisphere and the RF was located on bilateral hairy side of the mouth. The stimulation was performed on the contralateral side, from the center to the contralateral side of the mouth. The histograms are alignment at the moment when the experimenter touched the center of the mouth of the monkey, indicated by the light blue triangle. The end of the stimulation is indicated by the green triangles. The time between red dots and yellow circles indicates the baseline, while the time between light blue and green triangles indicates the stimulation epoch. The histograms are normalized for the maximal spike/sec value between the two conditions.*

One-hundred-eighthy-five of 223 neurons (82.95%) were classified as *Speed Unselective* neurons since they were modulated during the both conditions but they did not discriminate between them. The table S4.3 shows the identified RFs of the *Speed Unselective* neurons.

Forty-eight out of 185 (25.94% of *Speed Unselective* neurons) were inhibitory neurons since the firing rate of the stimulation epoch is significantly lower than the firing rate of the baseline, independently from the conditions. The figure S4.8 shows an example of *Speed Unselective* neuron.

These results show that a small set (N=38) of sensory task related neurons discriminated for the speed with which the passive touch stimulation was applied. Interestingly just one of 38 *Speed Selective* neurons showed higher neuronal modulation during the stimulation applied with the slow speed (1-5 cm/sec), while the remained 37 neurons showed selectivity for the fast speed stimulation (5-13 cm/sec).

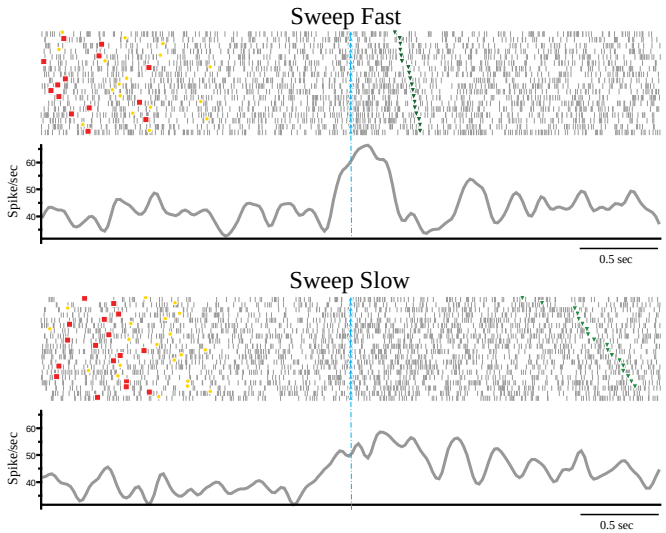


Figure S4. 8. *Example of Speed Unselective neuron. The neuron was modulated during both sweeping, independently from the velocity. The neuron was recorded in the right hemisphere and the RF was located on bilateral hairy side of the hand. The stimulation was performed on the contralateral side, from the distal (fingers) to proximal part (near wrist). The histograms are alignment at the moment when the experimenter touched the fingers of the monkey, indicated by the light blue triangle. The end of the stimulation is indicated by the green triangles. The time between red dots and yellow circles indicates the baseline, while the time between light blue and green triangles indicates the stimulation epoch. The histograms are normalized for the maximal spike/sec value between the two conditions.*

Relationship between speed of stimulation and firing rate

The categorization of the task related neurons in the two neuronal population, *Speed Selective* and *Speed Unselective* , was obtained from the first ANOVA performed into account two tasks with which the neurons were tested during recording session. Nevertheless, the applied velocity in each of the two tasks was not fixed for each trial, instead the speed varied from 1 to 5 cm/sec during Sweep Slow and from 5 to 13 cm/sec during Sweep Fast. Therefore, the applied speed of each trial in each of the two conditions had a specific velocity.

In order to more precise investigate the relation between the firing rate of each trial and the real applied velocity, we performed a correlation analysis considering for each trial the firing rate (spike/sec) during stimulation epoch and the speed (from 1 to 15 cm/sec), independently from the condition.

The analysis underlined that the 38 *Speed Selective* neurons have linear correlation between the speed of stimulation and the neuronal discharge (spike/sec). The 37 *Fast Selective* neurons had direct linear correlation while the *Slow Selective* neuron had inverse linear correlation. Therefore, higher is the speed, in the range 1-15 cm/sec, higher was the firing rate of the *Fast Selective* neurons and lower was the firing rate of the *Slow Selective* neuron.

Among the *Speed Unselective* neurons, the majority of them (N=164; 88.65% of the 185 *Speed Unselective* neurons) did not show any correlation. Nevertheless, a small percentage (N=21; 11.35% of the 185 *Speed Unselective* neurons) of *Speed Unselective* neurons showed direct linear correlation.

The results underscored that all the *Speed Selective* neurons and a small percentage of *Speed Unselective* neurons showed correlation between the firing rate during the stimulation and the speed with which the stimulation was applied, if in the range of 1-15 cm/sec. Therefore, their activation depended on the speed of the applied stimulation, since it increased from the lowest speed of the Sweep Slow condition to the highest speed of the Sweep Fast condition. The unique Slow selective neuron showed inverse linear correlation, therefore its activity decreased from the lowest speed in the Sweep Slow condition to the highest speed in the Sweep Fast condition.

Concerning the *Speed Unselective* neurons, even they did not discriminate the two sweepings because they were modulated during the stimulation epochs independently from the condition, a small set of those neurons showed correlation between the speed of stimulation and the firing rate. Since the *Speed Unselective* neurons did not discriminate the two sweeping conditions but their firing rate correlated with the velocity, one could supposed that for those neurons the two selected speed ranges were not efficient to identify the selectivity. Therefore, the first analysis underlined

that those neurons were not differently modulated if the sweeping was applied with the speed of 1-5 cm/sec, but the analysis of the firing rate of neuron during the sweep performed with a specific speed, *i.e.*.. trial by trial, it was possible to highlight that higher was the applied speed (from 1 to 15 cm/sec), higher was the firing rate. The correlation analysis revealed that those neurons could discriminated the speed, while the first analysis failed to find out the selectivity considering two range of velocity.

The figure S4.9 shows an example of *Speed Unselective* neuron that did not show the correlation between speed of applied stimulation and the firing rate, while the figure S4.10 shows an example of *Speed Unselective* neuron showing the correlation.

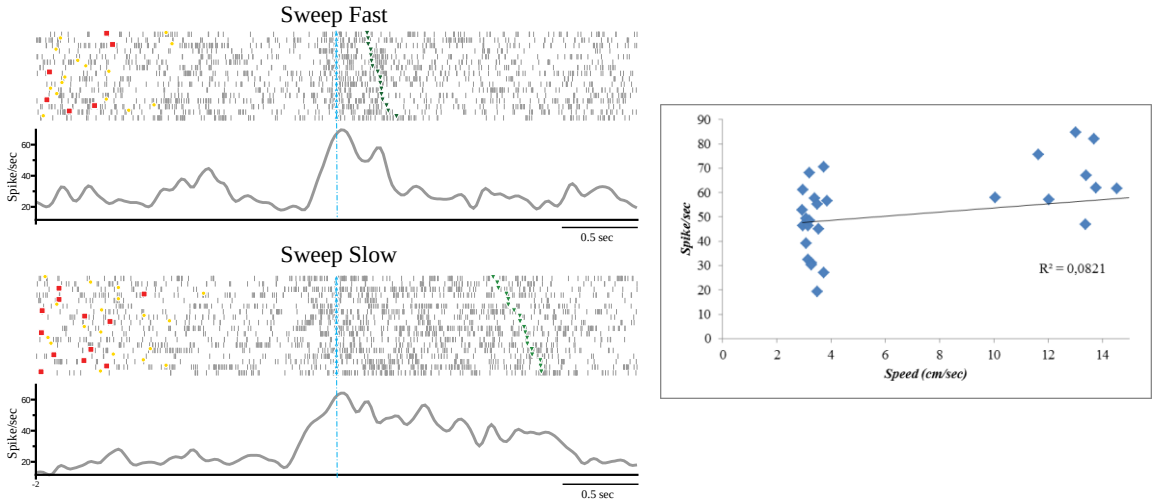


Figure S4. 9. *Example of Speed Unselective neuron without correlation between speed of sweeping and firing rate. The neuron was modulated during both sweeping, independently from the velocity.* ***On the left****: the histograms show that the neuron was activated for all the length of both the Sweep fast and the Sweep slow. Therefore, the correlation analysis confirmed that the modulation of neuron during sweeping did not depended from the applied speed. The neuron was recorded in the right hemisphere and the RF was located on bilateral forearm. The stimulation was performed on the contralateral side, from the distal (near wrist) to proximal part (near elbow). The histograms are alignment at the moment when the experimenter touched the fingers of the monkey, indicated by the light blue triangle. The end of the stimulation is indicated by the green triangles. The time between red dots and yellow circles indicates the baseline, while the time between light blue and green triangles indicates the stimulation epoch. The histograms are normalized for the maximal spike/sec value between the two conditions.* ***On the right:*** *the correlation between speed of stimulation (X-axis) and the firing rate (spike/sec, Y-axis) for the represented neuron. The graph shows the R-square value for the neuron. N.S. = not significant correlation.*

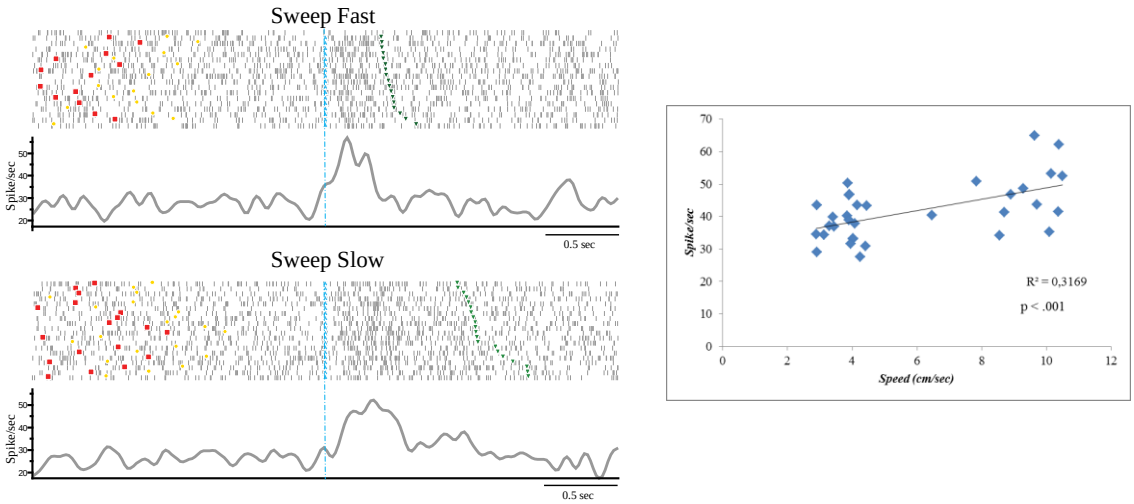


Figure S4. 10. *Example of Speed Unselective neuron with correlation between speed of sweeping and firing rate. The neuron was modulated during both sweeping, independently from the velocity. The correlation analysis underlined that the modulation of this neuron during sweeping depended on the applied speed.* ***On the left:*** *the histograms show that the neuron was activated for all the length of the Sweep fast and for a part of the Sweep slow. Since the centimeters of the stimulated RF were the same, the dropt of activity before the end of the Sweep slow was not due to the length of stimulated RF, instead to the applied velocity. The comparison between the two conditions, that took into account the two baseline and the two stimulation epochs, did not highlight the difference between the two but the analysis of the relation between speed and firing rate for each trial showed that higher was the speed, higher was the activation of the neuron. In fact the correlation analysis took into account just the firing rate of the stimulation epoch, without considering the baseline, neither the two conditions (Sweep fast and Sweep slow). The neuron was recorded in the right hemisphere and the RF was located on bilateral forearm. The stimulation was performed on the contralateral side, from the distal (near wrist) to proximal part (near elbow). The histograms are alignment at the moment when the experimenter touched the fingers of the monkey, indicated by the light blue triangle. The end of the stimulation is indicated by the green triangles. The time between red dots and yellow circles indicates the baseline, while the time between light blue and green triangles indicates the stimulation epoch. The histograms are normalized for the maximal spike/sec value between the two conditions.* ***On the right:*** *the correlation between speed of stimulation (X-axis) and the firing rate (spike/sec, Y-axis) for the represented neuron. The graph shows the R-square value for the neuron. The correlation is significant for* $p < .001$.

However, the percentage of *Speed Unselective* neurons showing above mentioned correlation is small in comparison to the unselective neurons that did not show the correlation. In order to investigate if the 21 neurons represented the real probability that a somato-sensory neuron that did not discriminate the two range of selected speed in the present study, *i.e...* 1-5 and 5-15 cm/sec, coded instead a specific velocity, we performed the chi-square test (χ^2). The cross table (2 x 2) was designed considering the two categories of neurons: Speed Selective and Speed Unselective with correlation and the number of neurons with correlation versus the number of neurons expected to have the correlation for each of the two categories. Therefore 38 *Speed Selective* and 21 *Speed Unselective* neurons had correlation, and we expected that all the 38 *Speed Selective* and 0 *Speed Unselective* neurons had correlation.

The results of chi-square test in the cross tabulation showed that there is the 19.02% of probability that a *Speed Unselective* neuron shows the correlation (χ^2= 17.26; *df*=1; *p*<.001). This result underscored that the probability is very small, therefore we could consider the *Speed Unselective* neurons with speed/firing rate correlation as a rare group of Speed Unselective neurons. A reason of the presence of correlation in absence of difference among the two conditions could be that the selected ranges could not be adequate to identified the selectivity for the speed for those neurons.

Moreover, in order to investigate the possible similarity between the correlation of *Speed Selective* and *Speed Unselective* neurons, we analyzed the distribution of R-square in the two categories. The Mann-Whitney U test result underlined that the R-square of the *Speed Selective* neurons (median=0.33) is statistically higher (*p*<.001) than the R-square of the *Speed Unselective* neurons (median=0.18). The figure S4.12 shows the distribution of R-square in the *Speed Selective* and *unselective* neurons. The R-square value was inside the range of 1) 0.1-0.2, of the 71.4% of the *Speed Unselective* neurons and of just 23.7% of the *Speed Selective* neurons; 2) 0.2-0.3, of the 19% of the *Speed Unselective* neurons and of 15.8% of the *Speed Selective* neurons; 3) 0.3-0.4, of the 28.9% of the *Speed Selective* neurons; 4) 0.4-0.5, of the 18.4% of the *Speed Selective* neurons; 5) 0.5-06, of the 9.5% of the *Speed Unselective* neurons and of 7.9% of the *Speed Selective* neurons; 6) 0.7-0.8, of the 5.3% of the *Speed Selective* neurons.

Interestingly, the *Speed Selective* neurons showed a wider distribution of R-square from 0.2 to 0.8, while the majority of *Speed Unselective* neurons showed the R-square inside the range 0.1-0.2. The results underlined that the correlations of *Speed Selective* and *Speed Unselective* neurons were different and stronger in the *Speed Selective* neurons, since the distribution of the R-square was significant higher than the R-square distribution of the *Speed Unselective* neurons. The figure S4.11 shows the distribution of R-square in the two categories, *Speed Selective* and *Speed Unselective*

neurons with correlation, while the figure S4.12 shows the percentage of Speed Unselective neurons showing correlation in comparison to those that did not showed the correlation.

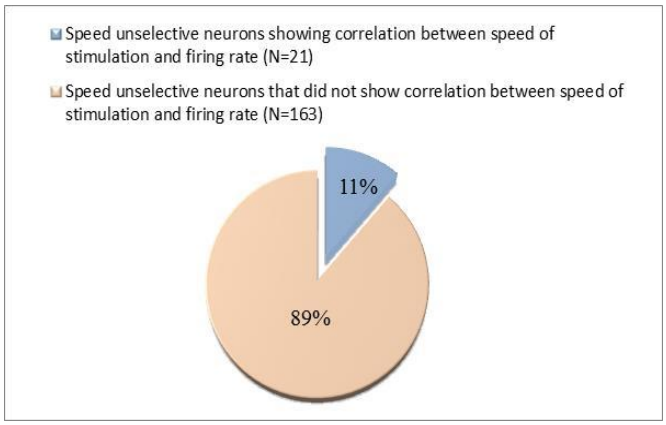


Figure S4. 11. *Percentage of Speed unselective neurons showing correlation between speed of stimulation and firing rate (11%) and Speed unselective neurons that did not show correlation between speed of stimulation and firing rate (89%).*

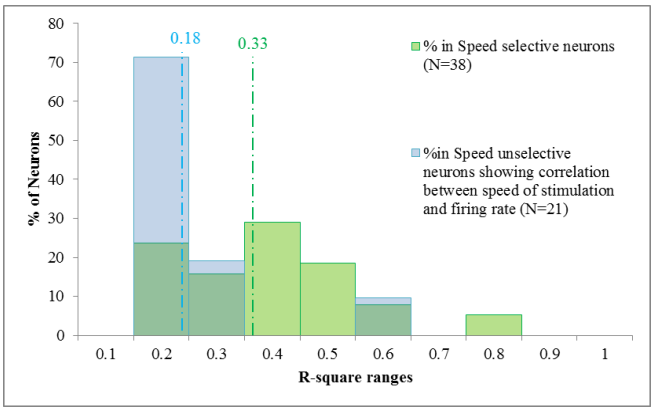


Figure S4. 12. *Distribution of R-square values of Speed selective and unselective neurons showing significant correlation between speed of the stimulus and firing rate. The dashed lines indicate the median value for Speed selective (in green) and Speed unselective neurons (in blue).*

Codification of speed in the three neuronal populations

The population analysis was performed on the three neuronal categories, *Speed Selective* , *Speed Unselective* presenting the correlation between firing rate and speed of stimulation and *Speed Unselective* without the correlation between firing rate and speed of stimulation. For this analysis we did not considered the *Slow Selective* neuron in the population of *Speed Selective* neurons.

The aim of the analysis was to investigate the profile of neuronal modulation in the two tasks, at population level.

The figure S4.12 A, B and C show the results of population analysis. The profile of activation of the population of the Speed Selective neurons (Fig. S4.12A) underscored that they were activated during both sweeping but the activation was higher during Sweep Fast than Sweep Slow from 80 ms after the alignment until 280 ms. The modulation during Sweep Slow started at the alignment and became statistically different from the modulation during Sweep Fast after 80 ms. Therefore the

codification of speed was significant after 80 ms from the beginning of the stimulation. Once coded the speed of applied stimulation after 80 ms from the alignment, if the speed is between 5 and 15 it will be the increment of the activity for all the length of the sweeping. Instead, if the speed was lower than 5 cm/sec, the modulation did not increase but remained stable and significantly lower until 280 ms. After 280 ms the activity during Sweep Fast and slow dropped down and they did not differed each others. The population was composed by neurons activated during both conditions but the activity during Sweep Fast was higher than during Sweep Slow and neurons modulated just during the Sweep Fast. The population profile underlined that the *Speed Selective* neuron population coded the fast speed. Therefore even at single neuron level the modulation was present also during Sweep Slow, at population level the modulation was significant just during the Sweep Fast.

The figure S4.12B shows the profile of activation of the population of the *Speed Unselective* neurons showing correlation between firing rate and sweeping speed. Likely the Speed Selective population, the neurons were modulated during both conditions from the alignment, but the two tasks differed each other in different period along the stimulation. In particular, the firing rate during Sweep Fast was significantly higher than during Sweep Slow after 40 ms from the alignment to 220 ms. On the contrary, the Sweep Slow stronger modulated the firing rate that the Sweep Fast from 460 ms to 680 ms after the alignment.

Moreover from the profile of modulation it is possible to notice that the modulation during Sweep Fast and slow started from the alignment but unlike the Speed Selective population the activity did not dropped own at similar timing. Instead during Sweep Fast the activity dropped down approximately as the activity during Sweep Fast of the Speed Selective population. During Sweep Slow the activity remained stable until all the length of the condition. Therefore, the population was modulated during both conditions for all the length of stimulation, without any differences between the two, from the alignment to after 2 sec. Nevertheless it is possible to underscore graphically the differences in the codification of the two conditions.

The figure S4.12C shows the profile of activation of the population of the Speed Unselective neurons without correlation. Likely the population of the Speed Unselective neurons with correlation, it was modulated during both conditions for all the length of stimulation, without any differences between the two, from the alignment to after 2 sec. Moreover the two conditions were coded modulation during the two conditions did not showed any difference even graphically, unlikely the population of Speed Unselective neurons with correlation.

The population analysis underscored that the three population differently coded the speed of applied stimulation and just the Speed Selective population selectively coded the fast speed. The population of Speed Unselective neurons without correlation did not differently coded the two

conditions and from the graph of profile it is possible to highlight that the curve of modulation is the same. The population of Speed Unselective neurons with correlation represented an intermediate population between the Speed Selective and Speed Unselective without correlation populations, since they did not differently coded the speed of stimulation, but graphically it is possible to identified that their modulation during the two conditions was present and remained stable from the beginning of the stimulation to the end, differently during Sweep Fast and Sweep Slow.

In fact this population consisted of neurons that showed correlation between speed and firing rate even they did not differently coded the two ranges of speed selected in the present experiment. The population graphic reflects the correlation since it is possible to discriminate the two conditions, therefore the different velocity even just graphically and not statistically. The graph of the Unselective population without correlation underlined that the modulation was independently from the speed.

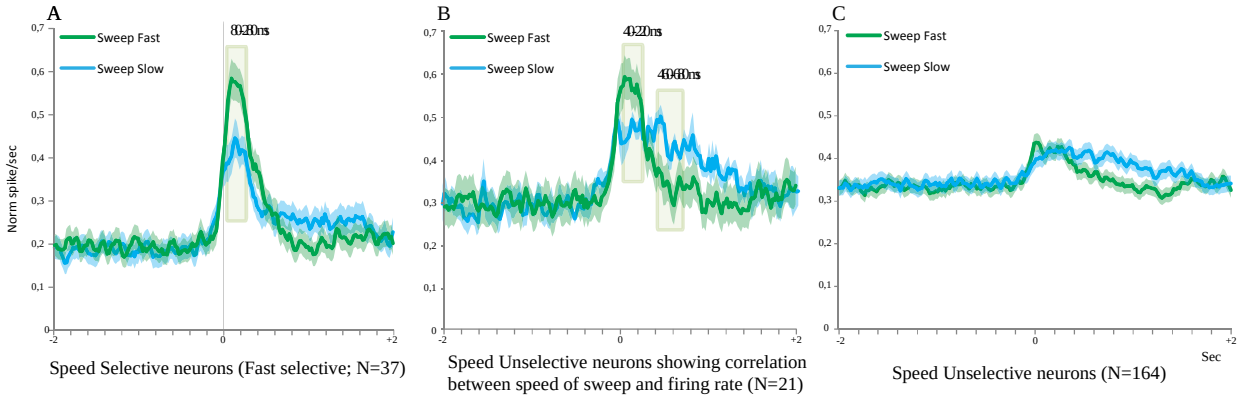


Figure S4. 13. ***A*** *Population of Speed Selective neurons activated stronger during Sweep Fast compared with Sweep Slow, in particular from 80 ms after alignment to 280 ms.* ***B*** *Population of Speed unselective neurons showing correlation between speed of stimulation and firing rate. From 40 ms after alignment to 220 ms, the population is activated stronger during Sweep Fast than Sweep Slow, while from 460 ms to 680 ms, the activity is higher during Sweep Slow than during Sweep Fast.* ***C*** *Population of Speed unselective neurons that did not show correlation between speed of sweeping and firing rate. The population does not discriminate the speed of stimulation and the 2 tasks do not differ each other. In* ***A****,* ***B*** *and* ***C****, the activity is alignment when the experiment touched the monkey's body. In* ***A*** *and* ***B*** *the light green shaded regions indicate the period in which paired samples t-tests evidenced a significant separation of the two curves (p<0.05).*

Primary somatosensory cortex does not code the speed of sweeping stimulation

We recorded a total of 29 task related neurons from both left and right SI (N=13 in the right hemisphere; N=16 in the left hemisphere). The identified RFs where the sweeping was applied were the contralateral mouth (N=15; 51.72%), the contralateral hand (N=11; 37.93%) and the contralateral check (N=3; 10.35%).

Among the 29 task related neurons, just 1 was *Speed Selective* (Fig. S4.14) . The remained 28 neurons were *Speed Unselective* (Fig. S4.15) . The correlation analyses underlined that the *Speed Selective* neuron and 3 of 28 *Speed Unselective* neurons showed linear correlation between speed of stimulation and firing rate. The majority of *Speed Selective* neurons (N=25) did not show correlation between speed of stimulation and firing rate.

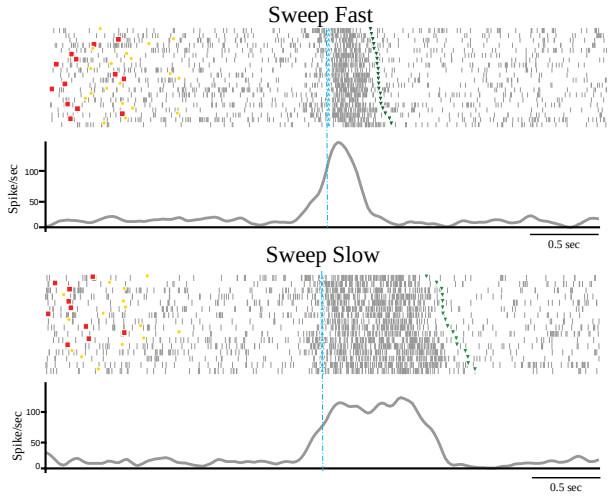
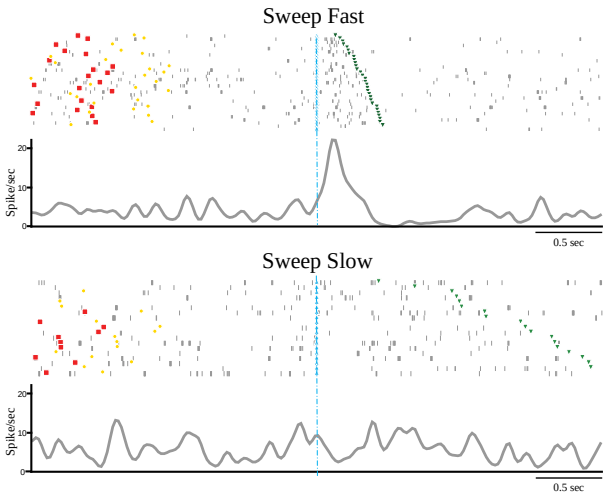


Figure S4. 14. *Example of Speed Unselective neuron recorded in the primary somatosensory cortex. The neuron was modulated during both sweeping, independently from the velocity. The neuron was recorded in the right hemisphere and the RF was located on contralateral mouth. The histograms are alignment at the beginning of the stimulation, indicated by the light blue triangle. The end of the stimulation is indicated by the green triangles. The time between red dots and yellow circles indicates the baseline, while the time between light blue and green triangles indicates the stimulation epoch. The histograms are normalized for the maximal spike/sec value between the two conditions.*

Figure S4. 15. *Speed Selective neuron recorded in the primary somatosensory cortex. The neuron was modulated during Sweep Fast condition. The neuron was recorded in the right hemisphere and the RF was located on contralateral fingers. The histograms are alignment at the beginning of the stimulation, indicated by the light blue triangle. The end of the stimulation is indicated by the green triangles. The time between red dots and yellow circles indicates the baseline, while the time between light blue and green triangles indicates the stimulation epoch. The histograms are normalized for the maximal spike/sec value between the two conditions.*



Conclusion and Discussion

Because of the key role of the posterior insula in the coding of affiliative interpersonal touch in humans (Olausson *et al.*, 2002, 2010), and its hypothetical involvement as a neural substrate of the social behavior in non-human primates (Kling and Stein, 1976), here we investigate for the first time the modulation of the disgranular and granular portions of insula of macaque monkey during a dynamic tactile stimulation, considered pleasant for non-human primate, *i.e.* the sweeping.

The sweeping is the common dynamic hand action that monkeys perform during grooming to move the fur and see the skin sites to be picked in order to remove ectoparasites or vegetation trapped. Even the primary hygienic function, it was also hypothesized that the grooming has a social role in the creation of alliances against common enemies.

Human studies demonstrated that the interpersonal pleasant touch activates the slow C-tactile low threshold mechanoreceptors (called CT fibers in human and C-LTMRs in other animals). CT fibers are low threshold mechanoreceptors, present on the hairy skin of many mammals and considered a specific coding channel for social touch occurring during close affiliative skin-to-skin interactions with conspecifics (Morrison *et al.*, 2010; Porges, 2003; Löken *et al.*,2009). CT fibers are modulated during dynamic tactile stimulation occurred on the hairy side of the skin with the velocity in the range of 1-10 cm/sec. The touch that activates the CT fibers is perceived as pleasant by subject who receive it, determines positive physiological effect, *e.g.* the decrement of HR, and modulated specific brain areas involved in the emotional codification of the peripheral somatosensory stimulation, as the insular cortex (Björnsdotter *et al.*, 2009). On the contrary, the CT fibers decrease their firing rate if the speed of stimulation is lower than 1 cm/sec or higher that 10 cm/sec. Moreover the tactile stimulation performed with a velocity outside the optimal range of 1-10 cm/sec is not perceived as pleasant, does not determined the activation of vagal system neither of the insular cortex.

Therefore the stimulation that activated the CT fibers is perceived as pleasant since modulated the parasympathetic nervous system, and it is coded as affiliative in the insular cortex.

Nevertheless there's no evidence about the modulation of these fibers during affiliative touch in monkeys. In a recent review Dunbar (2010) proposed that the soft touches arising from the sweeping might activate the CT fibers. In support of this hypothesis we demonstrated, in two above reported pilot studies (*Study 1 and Study 2*), that the sweeping among Rhesus monkeys is performed with a mean velocity of 9.31 cm/sec (*Study 1*), and that the human sweep determines the decrement of HR and increment of HRV, in a male Rhesus monkey (*Study 2*). Therefore the sweeping is performed with mean velocity inside the range that activates the human CT fibers and it determines

similar physiological effects, since the vagal modulation. Taken together, these data underscored that the sweeping are not performed with the unique aim to move the fur, but besides this merely hygienic function could be social affiliative reasons determined by activation of parasympathetic system by means the modulation of CT fibers. The non-human primates CT system could be therefore considered the analogous of CT system in humans. Since human studies (Olausson *et al.*, 2010; Morrison *et al.*, 2011) demonstrated that the pleasant touch that modulated CT fibers modulated the insular cortex, as above mentioned, in the present study we evaluated the role of the insular cortex in the codification of the sweeping in non-human primates.

Since the aim was to investigate the neuronal activity during a dynamic touch, we tested neurons with two randomly selected dynamic touches, the Sweep Slow and the Sweep Fast, both applied manually from the experimenter. During the Sweep Slow the experimenter moved the hand on the RF of the recorded neuron with a velocity of 1-5 cm/sec while during the Sweep Fast the applied velocity was 5-13 cm/sec. The velocities of the two stimuli were chosen based on the results of the *Study 1*,2 and 3 since we reported that the real sweeping is performed with a mean velocity of 9.31 cm/sec, and that the sweeping performed with the velocity of 5 and 10 cm/sec, but not lower that 5 and higher that 10 cm/sec, determined the positive physiological effects on monkeys, in terms of decrement of HR and increment of body temperature and HRV, therefore the activation of vagal system. In the present study we did not tested neurons with speed of sweeping higher that 15 cm/sec and lower than 1 cm/sec since these velocities of stimulation were uncomfortable for the monkey, as we already notice for the experiment of the *Study 2* and *Study 3* here presented, and as demonstrated from the increment of HR and decrement of HRV (*Study 2*). Moreover, the monkey started to struggle and rejected the stimulation after a while, and this would prevent the performing of the present study. We therefore decided to not test the neurons with those velocities.

We recorded a total of 223 task related neurons modulated during at least one of the two sweeping conditions. Since the tasks consisted of a passive dynamic stimulation, the task related neurons are considered a group of somatosensory neurons coding dynamic passive touch.

The identified RFs showed in the Table S4.1 were large, and bilateral. These characteristics are in line with the somatosensory properties of the recording areas, previously demonstrated (Ishida *et al.*, 2014). For each recorded neurons the stimulation was applied on the relative contralateral RF for all the length.

The task related neurons were divided in two categories, the *Speed Selective* and the *Speed Unselective neurons.* The *Speed Selective* neurons were modulated in one or both the two sweeping stimulation and one of the activity during one stimulation was significantly higher than the neuronal

modulation during the other one. The *Speed Unselective* neurons were modulated during both Sweep Slow and Sweep Fast and they did not show any statistical difference between the two.

Among the 223 task related neurons, 38 (17.04%) were *Speed Selective*, 37 of which were *Fast Selective* neurons since the modulation during Sweep Fast was significantly higher than the modulation during Sweep Slow. Just 1 of 38 was a *Slow selective* neuron since it was activated just during the Sweep Slow but not during the Sweep Fast condition. All *Speed Selective* neurons were excitatory neurons.

One-hundred-eighty-five of 223 neurons (82.95%) were *Speed Unselective* neurons since they were modulated during both Sweep Fast and Sweep Slow, but they did not discriminate between the two. Forty-eight out of 185 were inhibitory neurons, independently from the condition.

The selectivity of 37 of 38 *Speed Selective* neurons was for the Sweep Fast, while just 1 neuron showed selective for the Sweep Slow. One could speculated this findimg could be determined by the pressure adopted during the stimulation. In fact, even the experimenter was trained to perform the stimulation adopted the same pressure even with different velocities, the exact pressure was not determined online. Nevertheless, we could report here an example of neurons that did not support this hypothesis. The Figures S4.6 and S4.10 show the Speed Selective and Speed Unselective neuron, respectively. The two neurons were simultaneously recorded, therefore the adopted pressure was the same, but the selectivity was present just for one (neuron shows in the Fig. S4.6) of the two. Therefore this excluded the hypothesis that selectivity was determined by different pressure employed during the two sweeping. In fact if this were true, both neurons would have shown the selectivity.

The correlation analyses underlined that the 38 *Speed Selective* neurons have linear correlation between the speed of stimulation and the neuronal discharge (spike/sec). The 37 *Fast selective* neurons had direct linear correlation while the *Slow selective* neuron had inverse linear correlation. Therefore, higher is the speed, in the range 1-13 cm/sec, higher is the firing rate of the *Fast selective* neurons and lower is the firing rate of the *Slow Selective* neuron. This is in line with the expected, since the *Speed Selective* neurons coded the velocity of the applied stimulation. The Fast selective neurons were modulated selectively for the Sweep Fast and show linear direct correlation between firing rate and speed of stimulation. The *Slow Selective* neurons coded the slow speed and show inverse linear correlation.

The *Speed Unselective* neurons would not present correlation since they did differently coded the two conditions because they were modulated during the stimulation epochs independently from the condition. Nevertheless, the results were not as expected. In fact a small percentages (N=21; 11.35% of the 185 *Speed Unselective* neurons) of *Speed Unselective* neurons showed direct linear

correlation. Although, the majority of them (N=164; 88.65% of the 185 *Speed Unselective* neurons) did not show any correlation.

Since this group of Speed Unselective neurons did not discriminate the two sweeping conditions but their firing rate correlated with the velocity, one could presuppose that for those neurons the two selected speed ranges were not efficient to highlight the selectivity for a range of velocity. Therefore, the first analysis underlined that those neurons were not differently modulated if the sweeping was applied with a velocity in the range of 1-5 cm/sec or 5-15 cm/sec. Nevertheless, the analysis of the firing rate of each speed the highlight that higher is the applied speed, higher was the firing rate, from 1 to 13 cm/sec. The correlation analysis underscored the selective for the velocity that the first analysis did not. Another hypothesis could be that these neurons represent a category between the Speed Selective and Speed Unselective neurons. The neurons could detect a small variation of speed while the Speed Selective could detect bigger variation of speed, therefore they are able to discriminate if the velocity is inside a big range.

However, the percentage of unselective neurons with correlation is small in comparison to the unselective neurons that did not show the correlation. In order to investigate if the 21 neurons represented a case or a real probability that a somato-sensory neuron that did not discriminate the speed of stimulation shows the correlation, we performed the chi-square test (χ^2). The results of chi-square test in the cross tabulation showed that there is the 19.02% of probability that a *Speed Unselective* neuron shows the correlation (χ^2= 17.26; df=1; p<.001). This result underscored that the probability is very small, therefore we could consider the *Speed Unselective* neurons with speed/firing rate correlation as a rare group of Speed Unselective neurons.

Moreover, in order to investigate the possible similarity between the correlation of *Speed Selective* and *Speed Unselective* neurons, we analyzed the distribution of R-square in the two categories. The R-square of the *Speed Selective* neurons (Median of 0.33) is statistically higher than the R-square of the *Speed Unselective* neurons (Median of 0.18). Moreover, the R-square value of the majority of Speed Unselective neurons (71.4%) was inside the range 0.1-0.2, while the R-square value of the majority of *Speed Unselective* neurons (28.9%) was inside the range 0.3-0.4. Finally, the *Speed Selective* neurons but not *Speed Unselective* neurons showed a wider distribution of R square from 0.2 to 0.8.

Since the first analysis identified two population and correlation analysis underlined the presence of a third group inside a the Speed Unselective neurons, we performed the population analyses in order to investigate the profile of neuronal modulation in the two condition in each category.

The three populations showed different profiles (Fig. S4.13, S4.14 and S4.15). The profile of activation of the population of the Speed Selective neurons underscored that they were activated during both sweeping but the modulation during Sweep Slow became statistically different from the modulation during Sweep Fast after 80 ms. Therefore the codification of speed was significant after 80 ms from the beginning of the stimulation. Once coded the speed of applied stimulation after 80 ms from the alignment, if the speed is between 5 and 13 cm/sec it will be the increment of the activity for all the length of the sweeping. Instead, if the speed was lower than 5 cm/sec, the modulation did not increase but remained stable and significantly lower until 280 ms. After 280 ms the activity during Sweep Fast and slow dropped down and they did not differed each other's. The population profile underlined that the *Speed Selective* neuron population coded the fast speed. Therefore even at single neuron level the modulation was present also during Sweep Slow, at population level the modulation was significant just during the Sweep Fast. The activity during Sweep Slow, followed the timing of Sweep Fast and finished when Sweep Fast stimulation ended.

The profile of activation of the population of the *Speed Unselective* neurons showing correlation between speed of sweeping and their firing rate with underscored that the neurons were modulated during both conditions. The modulation during Sweep Fast was significant higher from 40 to 220 ms after the beginning of the stimulation. On the contrary, the modulation during Sweep Slow was significantly higher from 460 to 680 ms from the beginning of the sweeping. Therefore, even at single neuron level these neurons were modulated during sweeping, independently from the applied range of speed, the discrimination between the two conditions, Sweep Fast and Sweep Slow, emerges at population level.

Finally, the population of the Speed Unselective neurons without correlation between firing rate and speed of stimulation, was modulated during both conditions for all the length of stimulation, without any differences between the two, from the alignment to after 2 sec. Unlikely the population of Speed Unselective neurons with correlation, it was not possible to graphically discriminate the two conditions. The population was a somatosensory population modulated during dynamic touch independently from the applied speed.

In summary, the population of Speed Selective neurons coded the fast speed since they were not modulated during the entire length of Sweep Slow and the modulation during Sweep Slow lasted when the Sweep Fast finished and moreover the two conditions were significant different from 80 ms after the beginning of stimulation until 280 ms, the time of Sweep Fast. The population of Speed Unselective neurons showing the correlation between speed and firing rate did not differently coded at single neurons level the two range of speed but the firing rate depended on the speed of applied stimulation and the discrimination between the two conditions emerges and

population. The graphs of population analysis underscored that the modulation during Sweep Fast lasted at the end of fast stimulation, while during Sweep Slow lasted at the end of slow stimulation.

The Speed Unselective neurons could be involved in the codification of simple tactile stimulation that occurred in every action they performed. During a dynamic stimulation they were modulated since detect the deformation of the skin, independently from the type of stimulation. The Speed selective neurons, instead coded the speed of stimulation and in particular they were modulation if the Sweeping was performed with a velocity of 5-13 cm/sec but not lower. These neurons could be considered a specific type of sensory neurons involved in the codification of the dynamic fast touch. They were not usually modulated during simple touch that occurred in any moment and this determined the lower baseline level, since they were not usually involved in daily life. They were instead modulated during a specific touch, that must be occurred with a velocity that is characteristic of real sweeping among non-human primates (*Study 1* here) and that determines the relaxation of the monkey (*Study 2* here). The similar velocity of stimulation is characteristic of the pleasant touch among humans, *i.e.* the caress, that activated the insular cortex. Finally, the Speed Unselective neurons showing the discriminative properties between the two conditions at population level, but not at single neurons level and the correlation between their firing rate during sweeping and the applied speed, could represent an intermediate populations of neurons. They could be involved in both the simple touch and dynamic stimulation. At single neurons level they were activated independently from the applied speed, while at population level they discriminated between the two range of velocity.

Differently from the Speed Selective neurons that coded just the Sweep Fast, this population of Unselective neurons differently coded the Sweep Fast and the Sweep Slow.

We hypothesized that the Speed Selective neurons have a pivotal role in the codification of the dynamic touch performed with velocity in the range of 5-13 cm/sec since receiving information from the slow conduction unmyelinated CT fibers. We hypothesized in fact that the Sweep Fast may activated the CT fibers since the speed is in the range of the speed with which is performed the real sweeping among Rhesus monkey and the same speed modulated the vagal system. The sweep fast should reflect therefore the real sweeping motion. In accordance to the Dunbar's hypothesis above mentioned (2010), the stimulation may activated the CT fibers, that in turn should determine the modulation of the insular cortex, where it is coded as affiliative and pleasant (Olausson *et al.*, 2002 and 2008). On the contrary, the Sweep Slow was performed with a speed outside the range of velocity of real sweeping among monkey and that did not determined the positive modulation of parasympathetic system, instead the decrement of its modulation. Therefore the Sweep Slow should

be considered an unpleasant touch that is not coded as pleasant and affiliative in the insular cortex. The Sweep Slow should not activated the CT fibers.

In line with our hypothesis, the Speed Selective neurons should be modulated by the CT fibers, while the Speed unselective neurons should not. Nevertheless, one could criticized that the selectivity for the Sweep Fast observed in the Speed Selective neurons could be determined by the efference of fast adapting to secondary somatosensory cortex and insular cortex and not by the unmyelinated fibers. Since it is not possible to selectively activated the CT fibers by means of the Sweep manually performed, we performed a control experiment recording neurons in the primary sensory cortex using the same task. We recorded a total of 29 task neurons from both left and right primary somatosensory cortex. Among the task related neurons, 28 were Speed Unselective and just 1 was classified as Speed Selective. Even the small sample of recorded neurons in the primary sensory cortex as experiment of control, almost all of them did not discriminate for the speed of stimulation. Nevertheless, further studies will be necessary to deeply investigated the modulation of secondary somatosensory cortex and the insular cortex during the sweeping, in particular during the Sweep Fast and the activation of CT fibers during this kind of sweeping.

We suggest that the recoded task related neurons are a group of sensory neurons that are modulated during the dynamic passive touch similar to the human caress. The majority of the recorded neurons were unselective for the speed since they were modulated independently from the applied speed. A small percentage of the unselective neurons were modulated during both conditions and did not differently coded the selected speed range. Nevertheless their activity depended on the applied speed. A small percentage of task related neurons differently coded the two selected speed and higher was the applied speed, higher was their firing rate. Therefore this neuronal population coded the Sweep Fast instead the modulation during Sweep Slow was lower than Sweep Fast and did not covered the entire length of stimulation but finished as Sweep Fast time.

The present results underscored for the first time that SII, dIg and Ig of Rhesus monkey differently coded the speed of a passive dynamic touch, the sweep, common and affiliative touch for non-human primates. The sweep is similar to the human caress.

Human studies demonstrated that dynamic touch performed with a speed of 1-10 cm/sec is perceived as pleasant by the subject who receives it and by the activation of the slow-conducting unmyelinated C tactile (CT) afferents present on the hairy side of the skin, it determines 1) the vagal tone activation, therefore the decrement of the heart rate and an increment of the heart rate variability; and 2) modulation of mid- and posterior insular cortex.

Functional magnetic resonance imaging (fMRI) analysis conducted on two patients with a specific sensory neuronopathy that caused the complete loss of the large myelinated Aβ fibers, but have intact CT afferents, showed the modulation of mid- and posterior insular cortex during pleasant dynamic touch performed at a speed in the range of 1-10 cm/sec, but not of both primary and secondary sensory cortices. Human imaging studies of normal people underlined that the dynamic touch performed with a velocity of 1-10 cm/sec modulated the mid- and posterior insular cortex. The same results were obtained from patients with the sensory neuropathy that determined the absence on their skin of myelinated Aβ fibers in large skin areas, but with intact unmyelinated CT fibers, and from patients with the hereditary disorder, that determined the denervation of unmyelinated skin afferents (Olausson *et al.,* 2002. 2008 a.b.c; Löken *et al.,* 2009; Björnsdotter *et al.,* 2009). These findings supported the idea that the dynamic touch activates the CT afferents and these in turn modulate, by means of their projections inside the central nervous system, the insular cortex.

Our results are partly in line with these studies since we found that dynamic touch performed with velocities of 5-13 cm/sec modulated the insular cortex at single unit level. In addition our physiological data showed the presence of these kinds of neurons also in the secondary somatosensory cortex.

Even if human studies underlined the absence of the SII activation, from our preliminary results we cannot a priori exclude the involvement of SII in the codification of the affiliative sweeping. In fact, SII has connection with posterior insular cortex, has bilateral large RFs representation as insular cortex, in contrast to primary sensory cortex, and it is considered as a high order cortex involved in the integration and coding of the information received from primary sensory cortex.

Moreover, although human findings demonstrate that insular cortex is modulated by dynamic pleasant touch at 1-10 cm/sec of speed, our preliminary data showed that insula is differently modulated at single unit level in relation to touch performed at speed of 1-5 cm/sec (slow speed) or 5-13 cm/sec (fast speed), even if both velocities are inside the range of 1-10 cm/sec. The discrepancy between the human results that showed that the stimulation of 1-10 cm/sec activated the insular cortex, and our here presented results that showed that the stimulation of 5-13 cm/sec selectively modulated the insular cortex, could be explained by the different hairy skin properties of the monkey, that presents a huge fur in comparison to humans and the possible difference in the density of CT fibers.

Concerning the identified RFs in the three neuronal population, it could be explained by the position of recording chambers or by the hypothetical difference density of CT fibers in different

body parts. Even if so far there is no study in non-human primates, in mice (Liu *et al,*. 2007) it was demonstrated that these fibers are most densely distributed on the back. being sparsely present on the limbs. and completely absent on the paw skin. Moreover it was suggested (Walker *et al.,* 2014; Ackerley *et al.,* 2014) that the anatomical distribution of these nerve fibers determines people's emotional responses to touch depending on the interested body part. Based on these studies. we could hypothesized that the hands should have less CT fibers than the arms, in Rhesus monkey.

The high number of the hand and arm related neurons among Speed Selective neurons, could also be explained considering the sweeping sensory stimulation among free ranging monkeys, in naturalistic situations. The sweeping is a particular sensory stimulation occurred during allogrooming among monkeys. Allogrooming consisted basically of two motor actions: sweeping and picking of the fur. The sensory stimulation occurring during the sweeping is not just perceived by the recipient monkey, but also by the hand of the monkey agent of the grooming. Therefore we could speculate that the active sweeping sensory stimulation performed by hands is coded as positive and affiliative in the insular cortex of the agent monkey as the passive sweeping in the recipient monkey.

Taken together the data available up to now support the hypothesis that the sweeping motion could activate the CT fibers since is performed at the speed similar to that activating human CTs and determine the same positive autonomic effects evoked by the CT fibers activation in humans. It would be interesting to investigate if the coding of both fast and slow sweeping stimulation is compromised by a damage of the SII and/or insular cortex. In fact it was demonstrated that lesions of the insula (Kling and Steklis. 1976) led to the loss of grooming among Rhesus monkeys and of those neurons in the mid-posterior insular cortex that responded during hand-to-mouth motor actions such as grooming (pick and eat), picking somatosensory stimulation (grooming-like picking) and during observation of grooming in action (Grandi and Ishida, 2014). This evidence supports the data of the present study that demonstrated the coding of sweeping grooming in posterior insular.

The allogrooming is the most widespread and important social behavior among non-human primates. This behavior appeared in the first primates about 60 millions years ago and is present in Prosimians, Old world monkeys, New world monkeys and Hominidae. Therefore this behavior was likely present at the beginning of the primates evolution. Moreover, it is coded in insular cortex, that is an ancient brain region. As evolution proceeded, more complex connections with insular cortex have been generated, that expanded its role to the participation in socio-cognitive functions. In line with this point of view, the coding of a specific dynamic sensory tactile stimulation became typical of a specific social behavior.

Finally, even if the present results were obtained from just one monkey and this requires to be cautious in their interpretation, they can be considered as an important starting point in order to deeply investigate the pleasant sweeping stimulation at central nervous system level.

General conclusion: Sweeping, the monkey's pleasant touch

The sweeping is the common hand action that monkeys perform during grooming, a widespread behavior among mammals, birds and arthropods. There is two kind of grooming, the self- and the allogrooming. Among non-human primates, both self- and allogrooming are characterized by bimanual actions with rhythmic sweeps and plucking movements of the fingernails in precision grip, whilst being directed at addressing skin debris, spots, blemishes, ectoparasites or vegetation trapped in the fur (Tanaka, 1995), and for the control of lice infection (Zamma, 2002). In particular, self-grooming is directed toward the individual's own body, while allogrooming is carried out on others' body parts, inaccessible or invisible to self-grooming (Barton, 1985). Although the primary biological function of allogrooming is to take care of the body surface of others, many studies demonstrated its social function in many animals (Kimura *et al.*, 1996; Wilkinson, 1986; Böröczky *et al.*, 2013; Crowell-Davis *et al.*, 1986; Tyler, 1972; Rho *et al.*, 2007; Cox, 2012; Radford, 2008) and especially in non-human primates (Spruijt *et al.*, 1992). In fact, all non-human primates devote a significant amount of time grooming other individuals, suggesting that there is a reason behind this phenomenon, besides merely the hygiene function (Kummer, 1968; Boccia, 1989; Boccia *et al.*, 1989; Seyfarth, 1977; Kapsalis and Berman, 1996; Dunbar 1991 and 2010; Matheson and Bernstein, 2000; Tiddi *et al.*, 2011 and 2012; Maestripieri, 1993; De Waal, 2008; McFarland and Majolo , 2011; Schino *et al.*, 1988; Aureli *et al.*, 1999). It has been hypothesized that the allogrooming is the most common affiliative relationship and social strategy to create and maintain relationships and reliable alliances in order to respond collectively to whatever environmental, physical, social, or predatory challenges they may face (Dunbar, 2010; Maestripieri, 1993; De Waal, 2008; McFarland and Majolo , 2011). Moreover, it was reported that allogrooming enhance relaxation and feelings of security (Dunbar, 2010), while simultaneously reducing anxiety levels (Boccia *et al.*, 1989; Schino *et al.*, 1988). These effects were supported by the investigation of physiological parameters, such as heart rate (HR) and cortisol levels. In particular a decrement of the HR when receiving grooming (Boccia, 1989; Schino *et al.*, 1999), and a reduction of the cortisol levels during both passive (Gust *et al.*, 1993) and active grooming (Shutt *et al.*, 2007) was demonstrated.

Since grooming can have an affiliative quality (Kapsalis and Berman, 1996; Dunbar 1991 and 2010), it could be considered as the equivalent of the social interpersonal skin-to-skin contact of humans. More in detail, the sweeping could be considered similar to the human caress, since its characteristics. In fact, while the picking consists of a precision grip of the skin and the fur with nails and it could be quite painful for humans, the sweeping consists of the motion of the fur with a dynamic touch similar to the human caress. Nevertheless, even the human caress is commonly considered an affiliative interaction between people, the sweeping is performed for the unique aim

to move the fur. Nevertheless, new findings highlight the characteristics of sweeping that could be important in order to consider it as analogous to human caress for its physiological consequences. First of all, in a recent review Dunbar (2010) proposed that the soft touches arising from the sweeping may activate a class of slow unmyelinated C-tactile (CT) -afferent fibers. CT fibers are low threshold mechanoreceptors, present on the hairy skin of many mammals and considered a specific coding channel for social touch occurring during close affiliative skin-to-skin interactions with conspecifics (Morrison *et al.*, 2010; Porges, 2003; Löken *et al.*,2009). Human studies demonstrated that CT fibers are modulated during dynamic tactile stimulation occurred on the hairy side of the skin with the velocity in the range of 1-10 cm/sec and pressure of 0.2-2.6 nM. This touch is perceived as pleasant by subject who receive it, determines positive physiological effect, *e.g.* the decrement of HR, and modulated specific brain areas involved in the emotional codification of the peripheral touch, as the insular cortex. Therefore the stimulation that activated the CT fibers is perceived as pleasant since modulated the parasympathetic nervous system, and it is coded as affiliative at central nervous system level. This touch is commonly called caress or moderate massage and used in order to reduce depression and stress in patients and healthy people and also reduce the suffering of cancer patients receiving chemotherapy by means of positive effects in terms of heart rate modulation (Diego and Field, 2009).

Dunbar proposed the role of CT fibers during sweeping among non-human primates, nevertheless, up to now there is no direct evidence in support of this hypothesis, therefore 1) is the velocity of the sweeping among monkeys inside the range of 1-10 cm/sec, as affiliative caress in humans?; 2) are the effects of autonomous nervous system of the sweep similar to those of human caress?; 3) is insular cortex modulated, as during pleasant touch that activates the CT fibers in humans?

The aim of the present work was to answer to those questions, in support of the hypothesis, or not. The answers could allow to speculate the similarity of the monkeys pleasant sweeping with the humans gentle caress.

In particular, we investigated the 1) velocity of real sweeping among free ranging monkeys with a preliminary kinematic analysis from videotapes recording in a group of free ranging Rhesus monkeys (*Study 1*); 2) the effects on autonomic system, in terms of heart rate, heart rate variability of a male Rhesus monkey while receiving the sweep from experimenter, in a laboratory (*Study 2*); 3) the effects on autonomic system, in terms of body temperature changes of a male Rhesus monkey while receiving the sweep from experimenter, in a laboratory (*Study 3*); and 4) the codification of sweeping touch at central nervous system level in a male Rhesus monkey by means of single unit recording technique (*Study 4*).

The preliminary data here presented show that 1) the velocity of sweeping grooming touch among free ranging monkeys is in the range of the optimal velocity (1-10 cm/sec) to activate the C-tactile (CT) fibers in humans (9,31 cm/sec); 2) a sweep on the back of a male Rhesus monkey with velocities of 5 cm/sec and 10 cm/sec determined a decrement of the heart rate (HR) and increment of heart rate variability (HRV); 3) the sweep on the back of a male Rhesus monkey with velocities of 5-10 cm/sec determined the increment of the nose skin temperature, and 4) the sweep at velocity of 5-13 cm/sec determined the modulation of both insular and secondary somatosensory cortex.

These results support the Dunbar's hypothesis above mentioned related the involvement of CT fibers during sweeping grooming. In fact, this movement is performed at same velocity of pleasant human touch that activates CT fibers (1-10 cm/sec), determines the same positive physiological effects in terms of heart rate (decrement) and heart rate variability (increment) and the modulation of same brain regions (the insular cortex). Furthermore for the first times we investigated also effect of pleasant touch on the body temperatures, underlined that the pleasant sweep determined the increment of the nose skin temperature.

The present study represents the first indirect evidence of the hypothesis related the modulation of CT fibers system during pleasant sweeps, and of the representation of the affiliative gentle sweeping at both autonomic and central nervous system in non-human primates.

The preliminary data here presented highlight the similarity between human and non-human primates social touch system, even if we found some discrepancy with human studies.

In fact, the real sweeping mean velocity was of 9 cm/sec, almost the upper limit of range of speed that activated human CT fibers. Moreover, the positive autonomic effects, in terms of HR, HRV and nose skin temperature was obtained during the human sweep with a velocity of 5 and 10 cm/sec, therefore at the upper limit of the optimal range that activated the human CT fibers, while the sweep performed with a velocity lower than 5 cm/sec determined negative physiological effect. Human studies demonstrated that the optimal velocity is 1-10 cm/sec, while from our results the optimal velocity seems to be 5-13 cm/sec.

Finally, we found that not just the insular cortex has a role in the codification of the sweeping, as in humans, but also the secondary somatosensory cortex.

Taken together the results and the highlight discrepancies, underscore the homology between human and human CT system, and the necessity of further studies in order to deeply investigate the real sweep among monkeys, to more precisely determine the optimal velocity of the C-LTMR and to directly demonstrate their activation sweeping by means of their direct measurement. Studies in that direction will confirm the homology between human and non-human primate affective systems mediated by the CT fibers, therefore the similarity between humans' caress and monkeys' sweeping.

Moreover, the present study could be an important starting point to explore the evolutionary mechanism behind the transformation of the sweeping among non-human primates, utilitarian action during grooming to the affiliative caress among humans.

Finally, this investigation could add relevant information to be used not only for increasing our scientific knowledge, but also for better understanding how to use it for improving the well fare of experimental non-human primates.

In fact, recently Viktor and Annie Reinhardt (2008) have hypothesized that the positive physical contact with personnel could be a method to increase the welfare of single house cage experimental non-human primates. Nevertheless, even the general consensus of it, there is no published data to support this hypothesis. Human studies showed that the caress and moderate massages are utilized to improve the well-being not only for people suffering from depression, chronic pain, stress, neurological or psychological disease, and for cancer patients receiving chemo- and radio-therapy, but also to reduce the stress experienced by healthy people (Belinda *et al.* 2008; Billhult *et al.*2009; Diego and Field. 2009; Lindgren *et al.*,2010).

Therefore, the data here presented could be useful in order to reduce the stress under which experimental animals could be for experimental conditions, by means of human sweeping.

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