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Voluntary control of vocalization in the macaque ventral premotor cortex

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Introduction

Monkey Ventral Premotor Cortex

Architectonic organization of monkey ventral premotor cortex

The primate frontal lobe is subdivided into two main portions: the prefrontal cortex, located rostrally and the motor cortex, located caudally. The prefrontal cortex si considered to be implicated in planning complex cognitive behaviours, in personality expression, decision-making and moderating correct social behaviour. The motor cortex is defined as 'agranular frontal cortex' because it almost lacks granular cells of the IV layer as revealed from cytoarchitectonic investigations, and is involved in motor control.

The primate agranular frontal cortex has been subdivided by Brodmann (1909) into two cytoarchitectonically distinct areas: area 4 located in the precentral gyrus and area 6 located rostrally to area 4. Functionally, area 4 corresponds to ’primary motor area’, while the mesial surface (Penfield and Welch 1951; Woolsey et al 1952) of Brodmann's area 6 corresponds to 'supplementary motor area' and its lateral convexity is defined 'premotor cortex' (Fulton 1935).

Subsequent architectonic, connectional and functional studies in monkeys revealed that Brodmann's area 6 is made up of many structural and functional fields, each one processesing different aspects of motor behaviour.

Area 6 has been subdivided into three main regions: 1) supplementary motor area 'SMA proper' and area 'pre-SMA' on the mesial surface; 2) dorsal premotor cortex (PMd) on the dorsal convexity and 3) ventral premotor cortex (PMv) on the ventrolateral convexity.

PMv resulted to be an area not anatomically homogeneous: different architectonic investigations provided variable maps of PMv, which differed in terms of number, location and extent of these areas (Vogt and Vogt, 1919; Von Bonin and Bailey, 1947; Barbas and Pandya, 1987; Preuss and Goldmann-Rakic, 1991).

The studies of Matelli and co-workers (1985) provided a new detailed parcellation of the agranular frontal cortex. By adopting a chemoarchitectonic approach, they identified in PMv thwo distinct areas: area F4 which lies on the precentral gyrus, just rostral to the precentral area F1 (Brodmann's area 4) and area F5 located rostrally to F4 on the lateral cortical convexity and extending into the posterior bank of inferior limb (IAS) of the arcuate sulcus (Figura 1).

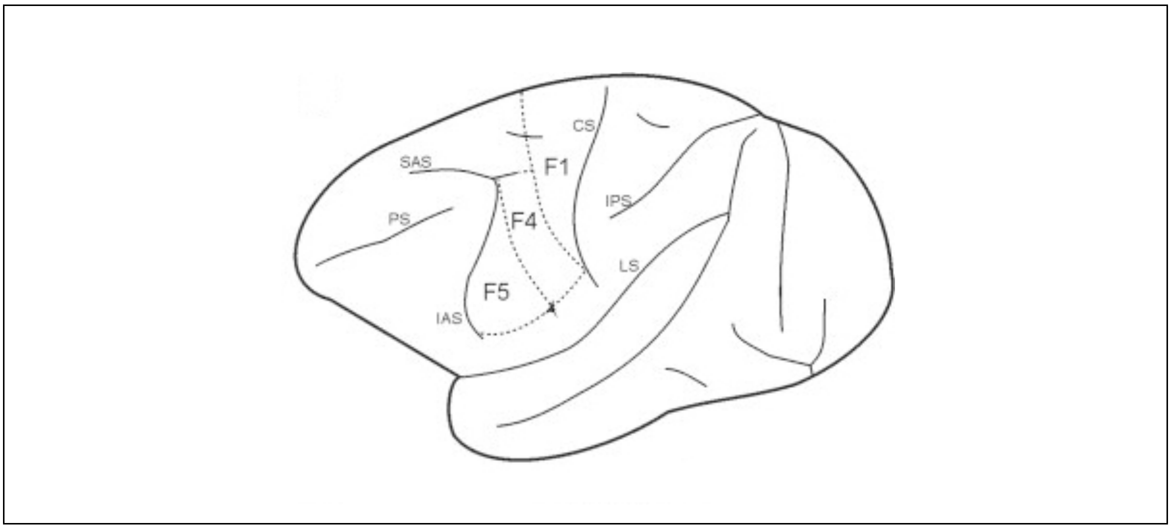

Figure 1. Architectonic map of the macaque PMv as proposed by Matelli et al. (1985)

A new study carried out by Belmalih and co-workers (Belamlih et al 2009) further subdivided area F5. It has been discovered that it is not homogeneous area consists of three architectonically distinct sub-areas: F5c that extends on most of the postarcuate convexity cortex immediately adjacent to IAS; F5p and F5a lie within the postarcuate bank at different medio-lateral levels. The present thesis refers to both Matelli's and Belmalih's parcellation.

Functional properties of the ventral premotor area F5

Over the last two decades, PMv has become the object of extensive functional investigations from single-neurons recording to functional imaging. Early electrophysiological studies have shown that the rostral part of PMv, corresponding to area F5, is involved in the control of hand and mouth movements (Rizzolatti et al 1981; Kurata and Tanji, 1986; Rizzolatti et al 1988; Hepp-Reymond et al., 1994). In the dorsal part of F5, buried in the posterior part of the postarcuate bank, neurons show changes in activity related to active hand movements (Rizzolatti et al 1981; Gentilucci et al., 1988). Electrical microstimulation of this F5 sector evoked fingers and wrist movements (Gentilucci et al., 1988). In its ventral part, lying on the postarcuate convexity cortex, neurons coding mouth movements tend to predominate (Gentilucci et al., 1988). In between these two distinct movements representation there is also a considerable overlap region in which both hand and mouth motor movements are coded (Ferrari et al., 2003).

Most of F5 motor neurons code the goal of motor acts such as object grasping, manipulating, holding or breaking. Usually neural activity starts before the effector-object contact and possibly ends either immediately after contact or continues through the next period. Some hand-related neurons present grip selectivity, being selectively active for precision grip, whole handgrip, finger grip etc. Other neurons do not differentiate between different effectors used, and can discharge when taking possession of a target is achieved by using the hand, the mouth or a tool tool (see Rizzolatti et al, 1996; Umilta' et al 2008).

More recent evidence indicated that F5p and F5c represent two functionally distinct rostral PMv subdivisions. One of the major differences between these two F5 sectors concerns the visual properties of the visuo-motor neurons, which have been subdivided into two main classes: 'canonical' and 'mirror' neurons (see Rizzolatti et al., 2000).

'Canonical neurons' are located in the posterior part of the dorsal part of posterior arcuate bank (Rizzolatti et al., 2000), in a position matching F5p as defined by Belmalih et al (2009). These neurons are typically active even when the monkey grasps an object with a particular grip, or when it observes three-dimensional visual stimuli compatible with this type of grip, even if in the absence of a motor execution (Rizzolatti et al., 1988; Murata et al., 1997; Raos et al., 2006). The properties of these neurons appear to reflect the results of a visuomotor transformation processing, leading to the selection of motor programs of distal movements for hand–object interactions (Jeannerod et al., 1995). Together, these data suggest for this F5 sector a relatively direct role in motor planning and execution of object-oriented hand motor acts.

'Mirror neurons' are typically located into the postarcuate convexity, namely area F5c (Belmalih et al 2009). Their properties are decribed in the next section.

Mirror Neurons

Mirror neurons have been initially identified as a class of visuomotor neurons active both when the monkey performs an object-oriented motor act and when it observes a similar motor act made by another individual (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996a). Extensive single neurons recording studies carried out in F5c area showed that they can code either hand, mouth or hand and mouth motor acts (Ferrari 2003). An effector-object interaction is needed to activate these neurons. Simple object presentation, or observation of an agent mimicking a motor act in the presence of an object, or simple biological movements (intransitive movements non object-directed) are not effective to induce neuron activation. Mirror neurons show a large degree of generalization regarding hand preference, direction of movement, and type of object target of the observed effective motor act. Furthermore mirror neurons can respond to the observation of a grasping motor act executed both by a conspecific and by a human experimenter, irrespective of the different visual aspect of the grasping hand. Although in the first description of mirror neurons, the distance at which the observed motor act was performed did not seem to matter, a recent study, specifically aimed to investigate this issue, demonstrated that the discharge of about half mirror neurons is modulated by the distance (peripersonal or extrapersonal) at which the observed motor act is performed.

All mirror neurons show a certain degree of congruence between observed motor acts they respond to and motor responses they code. According to the type of congruence they exhibit, mirror neurons have been subdivided into "strictly congruent" and "broadly congruent" (Gallese et al. 1996, Rizzolatti et at 1999a). The first class, represented by one third of the F5 mirror neurons, is active only for a precise type of observed grip (e.g. precision grip, whole handgrip, and finger grip). The remaining mirror neurons, in order to be triggered, do not require the observation of exactly the same motor act that they code motorically.

It has been proposed that the functional properties of mirror neurons reflect a mapping of the observed motor act onto one's own repertoire of motor representations coded in F5. These matching results would be at the basis of recognition and understanding of others' motor acts, a cognitive function relevant for interaction and communication in animals with a complex social behaviour, such as primates.

Early studies of mirror neurons concerned essentially the upper sector of F5 where hand motor acts are mostly represented. Recently, a study was carried out on the properties of neurons located in the lateral part of F5 (Ferrari et al. 2003), where, in contrast, most neurons are related to mouth motor acts.

Mirror Neuron System

Neurons responding to observation of motor acts done by others are present not only in area F5. Functional MRI (fMRI) data in the monkey showed that other prefrontal areas such as area 45A, 45B and 46 also respond to observing a grasping motor act (Nelissen et al 2005). Mirror neurons are also present in the rostral part of the inferior parietal lobule (IPL), particularly in area PFG (Gallese et al 2002; Fogassi et al 2005; Rozzi et al 2008) and the anterior intraparietal area (AIP) (Belmalih et al. 2009; Rizolatti et al., 2009). Both these areas are heavily connected with F5: PFG mostly with F5c, and the AIP with F5a (Rozzi et al., 2006; Borra et al., 2008). Both area PFG and AIP receive higher-order visual information from the cortex located inside the superior temporal sulcus (STS) (Rizzolatti et al 2009; Rozzi et al., 2006; Borra et al., 2008). STS areas encode biological motion, but they seem to lack motor properties. They cannot be considered therefore as part of the mirror system in a strict sense. Area AIP is also connected with the ventral bank of STS and the inferiotemporal cortex (Borra et al., 2008). This input could provide mirror areas with information concerning object identity. Finally, area F5 is connected with area F6 — the pre-supplementary motor area (pre-SMA) — and with the prefrontal cortex (area 46) (Rizzolatti et al 2001). The prefrontal cortex is also connected with AIP (Borra et al., 2008) and PFG (Rozzi et al, 2006). The prefrontal information could play a role in controlling the selection of self-generated and stimulus-driven motor acts according to the intentions of the agent (Fuster, 2008) (Figure 2).

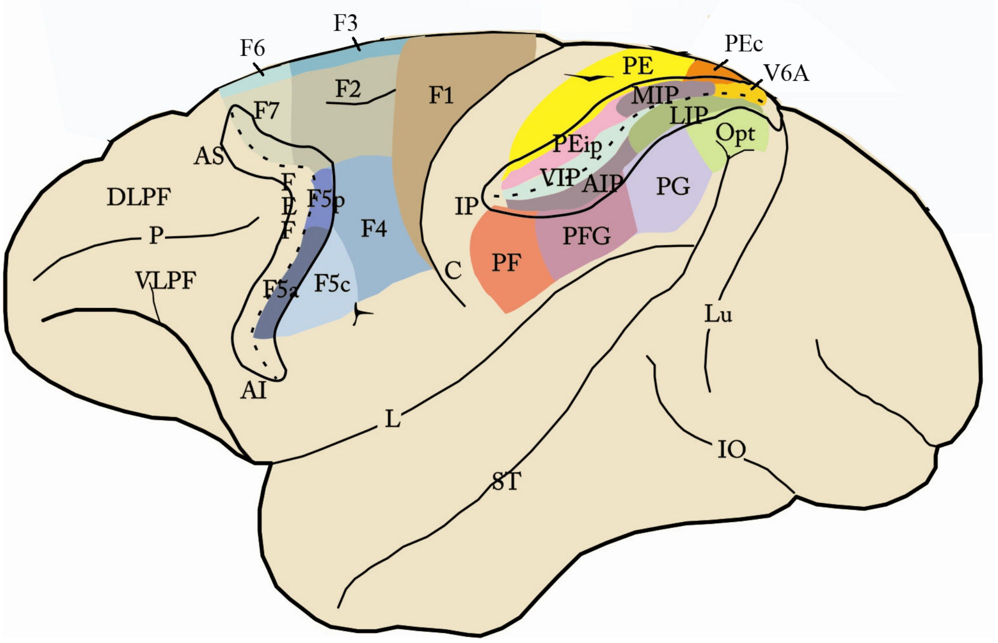

Figure 2. Lateral View of the monkey brain showing the parcellation of the Motor and Posterior Parietal Cortices. The areas located within the intraparietal sulcus and arcuate sulcus are shown on unfolded views of the sulci. For the nomenclature and definition of motor and posterior parietal areas, see Rizzolatti et al. (1998a). AI, inferior arcuate sulcus; AS, superior arcuate sulcus; C, central sulcus; DLPF, dorsolateral prefrontal cortex; VLPF, dorsolateral prefrontal cortex, ventral; L, lateral fissure; Lu, lunate sulcus; P, principal sulcus; ST, superior temporal sulcus.

It was recently shown that, in addition to areas PFG and AIP, two other areas of the parietal lobe contain neurons with mirror properties: the lateral intraparietal area and the ventral intraparietal area (Shepherd et al., 2009; Hishida et al., 2009).

The mirror system in humans

A parieto-frontal, Mirror System, has been found also in humans, based on evidence from electroencephalography (Muthukumaraswamy, et al., 2004), magnetoencephalography (Hari et al., 1998), transcranial magnetic stimulation (Fadiga et al., 1995), and functional magnetic resonance imaging (fMRI)

(Iacoboni et al., 1999; Buccino et al., 2001; see Rizzolatti and Craighero 2004). A reach amount of studies showed that observation of motor acts done by others activates, in humans, a complex network formed by occipital, temporal, and parietal visual areas, and two cortical regions whose function is fundamentally or predominantly motor (Decety et al. 2002; Grafton et al. 1996; Grèzes et al. 1998; Grèzes et al. 2001; Grèzes et al. 2003; Iacoboni et al. 2001; Koski et al. 2002, 2003; Manthey et al. 2003; Nishitani & Hari 2000, 2002; Rizzolatti et al. 1996b). These two last regions are the rostral part of the inferior parietal lobule and the lowest part of the precentral gyrus plus the ventral premotor cortex and the posterior part of the inferior frontal gyrus (IFG). These regions form the core of the human mirror-neuron system.

The mirror neuron system provides a basic mechanism that unifies motor act production and motor act observation, allowing the understanding of the motor acts of others from the inside. Such motor-based understanding seems to be a primary way in which individuals relate to one another.

Ingestive and communicative mirror neurons

Ingestive mirror neurons respond to the observation of motor acts related to ingestive functions, such as grasping food with the mouth, breaking it, or sucking. Neurons of this class represent about 80% of the total amount of recorded mouth mirror neurons. As described above, also ingestive mirror neurons need the interaction of the effector with the object and are not triggered by simple object presentation or motor act mimicking. Most of them present also strictly congruence aspects. Interestingly, a recent fMRI study showed that in humans there are specifica regions active during the to observation of ingestive motor acts. It has been observed that mouth motor acts, such as biting or chewing, activate bilaterally the posterior portion of the inferior frontal gyrus, particulary the opercular part (area 44) (Buccino 2001) This area is considered to be the homologue of monkeys area F5 (Rizzolatti and Arbib 1998) (See BOX 1).

A second group of mouth mirror neurons recorded in monkey is less numerous (about 20%). These mirror neurons are referred to as communicative mirror neurons (Ferrari et al. 2003). They are active in response to observation of communicative facial gestures belonging to a specie-specific context (Maestripieri, 1996; Van Hoof 1967) such as lipsmacking or tongue protrusion. Communicative mirror neurons have complex motor aspects: they show a strong discharge when the monkey actively performs an ingestive motor act. In this respect they behave as the ingestive mirror neurons. This discrepancy between the effective visual input (communicative) and the effective executed motor act (ingestive) could be solved if we consider some evolutionary hypotheses. There is evidence suggesting that communicative gestures, or at least some of them, derived from ingestive motor acts (MacNeilage 1998, Van Hoof 1967). From this perspective, it could be argued that the communicative mouth mirror neurons found in F5, reflect a process of corticalization of communicative functions not yet freed from their original ingestive bases.

The presence of communicative mirror neurons in a monkey motor area suggests that this area is involved in some rudimental aspects of social communication. The presence of these neurons in one area considered homolog to human Broca's area, seems to be in accord with the gestural theory of language evolution: a gestural communicative system evolved into a human linguistic communicative system.

Audio-visual Mirror Neurons

Recently, Kohler et al. reported that some neurons in monkey ventral premotor cortex (area F5), responding during execution and observation of motor acts, are active even when only the sound of the effective motor act is presented to the monkey. Other types of arousing sounds, such as white noise or monkey vocalizations, typically do not evoke significant responses in these neurons. (Kohler et al. 2002). These neurons are selective for different sounds produced by specific motor acts, such as breaking peanuts or ripping paper. For their characteristics they have been named 'audio-visual' mirror neurons.

Multimodal neurons responding to acoustic stimuli have been described in several cortical and subcortical areas including superior temporal sulcus region (Baylis et al 1987; Mistlin and Perrett 1990), ventral pre-motor cortex (Rizzolatti

1981; Graziano et al 1999;), and superior colliculus (Stein et al 2001). These neurons, however, responded to specific stimulus locations or directions of movement.

Unlike these neurons, audio-visual mirror neurons do not code space location of the sound or peculiar characteristics of it, but specifically respond to the sound of motor acts. These neurons take part to motor act planning and execution, as demonstrated by their motor response, and could represent a neural correlate of the mechanism allowing one to understanding noisy motor acts performed by others, even in the absence of a direct visual access. In other words, acoustic information is enough to evoke a motor content.

The abstract motor act representation embodied by audiovisual mirror neurons according to Keysers et al. (2003), is comparable to the way we use verbs in language: the verb ‘break’ is used to represent an abstract meaning used in different contexts: ‘I see you breaking a peanut’, ‘I hear you breaking a peanut’, ‘I break a peanut’. The verb, just as the responses in audiovisual mirror neurons, does not change depending on the context in which it is used, nor depending on the subject/agent performing the motor act.

What is intriguing about the discovery of audiovisual mirror neurons is that they are observed in an area that appears to be the homologue of human Broca’s area (area 44) (Petrides and Pandya, 1994; Rizzolatti and Arbib, 1998).

The capacity to auditory access motor act representations is very similar to characteristics of human speech. Furthermore, the property of these neurons to represent motor act contents and the area where they were discovered may shed light on the evolution of spoken language.

Cortical larynx area

It is difficult to define the complete cortical representation of the larynx because of the anatomical complexity of this organ, involved in several functions as vocalization, respiration and swallowing. As reported by Hast (1974), this topic has been interest of research since the end of the 19th century.

Major physiological and anatomical contribution to larynx cortical representation derives from stimulation studies carried out by Semon and Horsley (1890).

These authors demonstrated that electrical stimulation of the foot of the ascending frontal gyrus between the anterior subcentral sulcus (dimple) and the inferior limb of the arcuate sulcus produce abductory movements of monkey, cat and dog larynx.

Several others stimulation studies investigated different points within the central nervous system which can cause vocal cords movements. Leyton and Sherrington (1917) confirmed Semon and Hosley data in gorilla and chimpanzee. The same results were reported also by Sugar et al. (1948).

However, the first study exploring the whole area bounded anteriorly by the inferior limb of the arcuate sulcus and posteriorly by the lower limb of the central sulcus was performed by Hast (1974). The electrical stimulation of 14 anesthetized Rhesus monkeys lead him to topographically locate intrinsic and extrinsic larynx muscles representation, by observing that there are identifiable into sub-areas located within PMv (Figure 3).

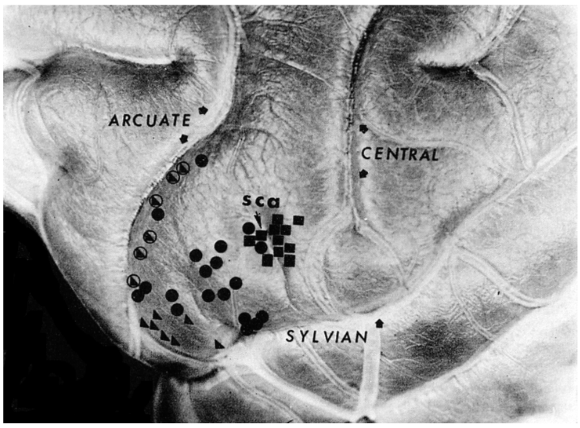

Figure 3. Pothograph of representative brain of *Macaca mulatta* illustrating specific foci for responses of both intrinsic and extrinsic lryngeal muscles: right-angled triangle and dots= intrinsic laryngeal muscles; black square= extrinsic Laryngeal muscles. sca= sulcus subcentralis anterior (subcentral dimple) (from Hast et al 1974).

Recently, neuroanatomical studies were carried out in macaque and squirrel monkeys to outline cortical connections of the primate larynx area (Simonyan and Jurgens 2003 and 2005). According to these authors, almost all cortical connections of motor larynx area are reciprocal. This area receives and projects heavily into the neighboring inferior premotor (where mouth mirror activity was observed), prefrontal (Brodmann area 44 and 45), primary motor cortex (tongue, lip and jaw representation), anterior cingulate cortex (known to be involved in vocal production in primates), secondary somatosensory cortex and insula. Weaker reciprocal connections exist with the dorsolateral premotor, the SMA, the primary somatosensory cortex and the cortex within the superior temporal sulcus. The only region projecting to the larynx area, without receiving an input from it, is the central orbital frontal cortex (area 13). Anterograde projections of the larynx area, that are not reciprocated, go to the inferior parietal cortex, the posterior parietoopercular cortex and the cortex within the intraparietal sulcus.

Also subcortical structures are connected with larynx motor area. Reciprocal connections were observed with the clastrum and the Meynert nucleus (important for attention control and memory processes). Furthermore basal ganglia, (putamen and caudate nucleus) and thalamus are involved (Simonyan and Jürgens 2004 and 2005).

The authors considered macaque and squirrel monkey as potential models in the neuroanatomical investigation of the human larynx area. However, crucial differences were reported between the functional representation of larynx in monkey and human cortex. Lesions data (Foix et al., 1926), stimulation experiments (Foerster, 1936; Penfield and Bordley, 1937) and neuroimaging investigation (Bookheimer et al., 2000; Perry et al., 1999) showed a minor involvement of precentral gyrus in human phonation control.

Simonyan and Jürgens (2005) observed that the lesion of the lateral precentral cortex in the monkey does not impair vocal communication (Kirzinger and Jürgens, 1982; Sutton et al 1974; Aitken 1981). They ascribed the motor cortical larynx representation to some voluntary control over vocal fold movements, but they also argued that this control possibly is non-vocal, being related, for instance, to abdominal straining movements carried out during large jumps, lifting heavy weights, defecation or delivery.

However, the presence of larynx motor neurons in an area in which hands and mouth motor control is represented and where mirror properties are involved could indicate a possible role of this area in the development of language, according to the gestural theory of language evolution (see next section).

Monkey Communicative Systems

Communication system in monkeys and the role of vocalization

While language in the strict sense may be uniquely human, numerous other species have their own way to communicate. Many of these communication systems share at least some properties with human language.

In the last few years we have witnessed a growing interest in animal social communication especially the one of the primates closer to us. This is due to further realization that primates can communicate not only information regarding their emotional state but also about the surrounding physical and social environment. (Maestripieri, 1996).

The concept of information plays a central role in studies of animal communication, but it is still an open problem how animals can share information. In particular, there is strong debate on which could be the crucial element underpinning animal communication, if vocalization or gesture (Corballis, 2003). Probably the conclusion closer to reality is that both aspects, gestural and vocal, are important and have a role in monkey communication.

Gestural communication

Communication can be defined as “transmission [of information] between a sender and a receiver through auditory, olfactory, tactile and visual signals and the consequent use of that information by the receiver to decide how to answer” (Pika, Liebel, Tomasello, 2003).

Several ethologists have conducted observational studies both on apes (for example de Waal, 2001: Pika, Liebal, Tomasello, 2003) and monkeys. Primates communicate using manual and body gestures mainly in close-range social contexts such as play, grooming, nursing and during sexual and agonistic encounters (Call and Tomasello, 2007). Many of them are fairly involuntary facial expressions or body autonomic responses (e.g. piloerection) but an important subset of these seems intentional or voluntary controlled by the individual. Communication in apes is considered more complex and presenting more potentials than monkeys one: for example, only in the first species iconic use of gestures and behaviours, such as pointing at, are produced voluntarily

(Leavens and Hopkins 1998).

Moreover, starting in the late 1960s, several apes of four different species (Chimpanzee, Bonobo, Gorilla and Orangutan) have been shown to be able to learn and reproduce a large number of communicative signs (Rumbaugh 1975; Savage-Rumbaugh et al 1986; Patterson et al 1990). Monkeys that are evolutionarily further from us seem to have less flexibility compared to apes (Tomasello and colleagues, 1997) in the use of communicative gestures (Maestripieri 1999). For example, spontaneous use of a pointing gesture has never been reported and to our knowledge there is no evidence of monkeys learning any type of sign language. However, with respect to communication among individuals, monkeys present a large repertoire of facial expressions with specific meanings and used in specific contexts. For example, Van Hooff (1967) created a classification of the main Catharine monkeys' facial expressions, analyzing similarities and differences among different species.

It seems that primate facial expressions represent evolutionary legacy (Thierry et al 2004). Old world monkeys use about seven displays in the context of bonding alone. (van Hooff 1967, Redican 1975 *and* Preuschoft and van Hooff 1995). These displays appear to have different social functions in different species. Many facial displays are ancestral homologies of all the macaques and are also shared with other Old World monkeys, with apes and sometimes even with New World monkeys (Preuschoft and van Hooff 1995) (e.g. relaxed open-mouth, and the silent bared teeth). However, the functions of these facial expressions vary among species ranging from formal subordination, over submission and appeasement, to affiliation, reassurance and even to playfulness. (Preuschoft 1992)

A research conducted by Maestripieri (1996) on the *Macaca nemestrina* species (which belongs to our experimental subject) identified 15 main behaviors and reported the following ones as more frequent:

- *Pucker face*
- *Lip-smacking face*.
- *Bared-teeth*
- *Present*

In this monkey species gestural signals appear to be used to communicate to other individuals emotional states and intentions, but also to request their participation in precise social interactions.

Vocal communication

Marler asks, *"What do animal sounds mean" ?* (Marler 1998).

Verbal language is considered different from non-human primate vocalization: the first is learned, under voluntary control, referential and grammatically organized, while the second has been described especially as genetically programmed, involuntary and strictly connected to the emitter's emotional state (Taglialatela, Savage-Rumbaugh and Baker, 2003). However, recent studies conducted on both free-range and captive primates suggest the dichotomy is less strong than thought before.

Seyfarth and colleagues (1980) demonstrated that monkeys use alarm calls and/or food calls that, for all practical purposes, function as if they carried symbolic referential meaning. Originally Seyfarth et al. (1980) studied alarm calls in vervet monkeys. These monkeys have a set of three distinct alarm calls used for three different predators (snakes, leopards and eagles). When an individual hears one of these calls it adopt an appropriate and specific motor strategy: run for cover in bushes when hearing the eagle call, climb up into the treetops when hearing the leopard call and stand up to scan the grass when hearing the snake call.

Since the original work by Seyfarth et al. (1980) similarly "functionally referential" calls have been observed in few lemurs (Ficthel 2004), squirrel monkeys (Zuberbühler, 2000) and chimpanzees (Byrne, 2000 and Crockford and Boesch 2003). The results of all these observations suggest that when recipients perceive a signal they acquire information, and the acquisition of this information, among other things, changes their behavior. That information is a simple integration of information acquired from calls and other contextual cues (Seyfarth et al. 2010)

Furthemore, various species learn to produce certain types of vocalizations in precise contexts and learning can also model the structure of some produced vocalizations (e.g. Hihara et al., 2003). For example, Taglialatela, et al (2003) worked with a bonobo who was able to produce distinct vocalizations, which varied systematically in relation to the semantic context and hypothesized that this vocal variability could have its own role in transmitting information, ‘meanings’ to conspecifics. Some flexibility in the precise manner in which a given call is produced was demonstrated also in Tamarin monkeys. Some Tamarins produce food calls when they discover food, but rates depend on whether or not other group mates are present (Caine, Addington and Windfelder 1995). Vervet monkey females adjust the rate of alarm calls depending on whether their own offspring is present whereas males call more often when female are present (Cheney and Seyfarth, 1985).

Zuberbühler reported, in different playback experiments, that Diana monkeys respond not only to their own alarm calls, but also to the ones of other species living in the same area, notably guinea fowls, chimpanzees and Campbell’s monkeys. Diana monkeys respond to these calls with appropriate motor acts (Zuberbühler, 2005).

Evidence that recipients are responding to the meaning of such calls and not to other aspects, such as emotional intensity, comes also from experiments in which individuals habituated to some calls show dishabituation only when the call’s meaning changes (Cheney and Seyfarth, 1988; Zuberbühler et al, 1999)

Despite all this evidence (referentiality, learning, comprehension etc.), there is currently a wide consensus that human language differs from animal communication in a profound way, because the essence of human language is its complex grammatical organization and generative power, something that is widely lacking in all animal communication systems (Chomsky 1981).

Recent studies have actually shown that non-human primates combine finite and stereotyped sound elements to form more complicated structures. Chimpanzees (Crockford and Boesch 2005), bonobos (Clay and Zuberbühler 2009) and also monkeys have been observed to combine spontaneous call. In putty-nosed monkeys, adult males produce two loud calls, ‘‘pyows’’ and ‘‘hacks,’’ in a range of contexts, including predation. However, when combining the two calls in one specific way (i.e., a few pyows followed by a few hacks), males give a supplementary message to their group members to move away from the current location (Arnold and Zuberbühler 2006, 2008). Free-ranging Campbell's monkeys are able to combine a repertoire of six call types into nine distinct call sequences (Ouattara et al. 2009). These call combinations are not random, but the product of rules aimed to obtain a precise semantic content.

The existence in primates of a certain degree of vocal flexibility, as demonstrated by monkeys ability to use calls in different sequences (see Tomasello and Zuberböhler 2002), together with the demonstration of an embrionic form of functionally referential vocalizations, could be the key of the evolution of a more complex communicative form such as human language.

However, it is important to remember a fundamental difference between human linguistic system and non-human primates communicative vocal system: there are no convincing proofs that non-human primate vocalizations refer to past or future facts or events: they refer to current events or objects.

Language Origins

Language Origins

Fossil evidences and genetic research established, beyond reasonable doubt that we, with all our advanced cognitive and linguistic abilities, have evolved from ape-like creatures. Both vocal and gestural communication systems in non-human primates seem to have some rudimental properties of human language, however the verbal language is what distinguishes humans from other animals. A question that linguistic society, psychology, ethology and neuroscience researches are attempting to answer is: how did such special human ability evolve? Did language first evolve in the spoken modality dominant today or was another modality, presumably gestures, used in the early stages? If language did first evolve in a gesturing modality why did we switch to speech?

Vocal Theory of language evolution

Let us consider first what might be called the vocal theory of language origin. Some primatologists and cognitive ethologists (e.g., Ghazanfar & Hauser, 1999; Snowdon 1997) maintain that strong similarities exist between primate vocal behaviors and human speech. As reported in the Monkey's vocal communication section, there are several evidences that also non-human primates are able to use vocal production as functionally referential (Seyfarth & Cheney, 1997; Dittus, 1984; Gouzoules et al., 1984). In addition behavioural studies (for example Hauser and Andersson 1994; Le Prell et al 2002), lesion studies (Heffner and Heffner, 1986) and a PET imaging studies (Poremba et al 2004) demonstrated that conspecific vocal processing in macaques, like humans, is biased towards the left hemisphere.

According to Arcadi (2005), all these data suggest that some of the substrate on which language is based can be explored through comparative analyses of the vocal behaviour of our closest phylogenetic relatives.

Supporters of this theory believe that about 100,000 years ago, certain changes in mouth and pharynx combined with an increased brain volume gave human beings voluntary control over vocal outputs. Some authors speculate that human language arose from the development of onomatopoeia: imitation of sounds in the environment. According to another hypothesis, the use of arbitrary symbolic sounds developed from the cries primates used to alert one another to the presence of predators, edible or poisoning foods, and so on. What is, in general, supported by the vocal theory of language evolution is that words first and sentences later are the results of evolved original monkey vocalizations submitted to natural and social selective influences (Arcadi, 2000). An integration of vocalization and manual gestures, also according to Charles Darwin (1871), could have happened later.

Gestural Theory of Language evolution

Another school of thought posits the gestural theory of language origins.
The idea that language evolved from manual rather than vocal gestures is often attributed to the 18th-century philosopher Condillac [1746]. In non-human primate communicative there is evidence of a good intentional control of upper limb and facial expressions but these species lack a fine flexibility in vocal control. This lack of a complete control over the vocal system is present also in apes, as evidenced by the failure in teaching apes to talk while some form of signs symbolic language is properly used. A famous example very often cited is that of Kanzi, a bonobo who unexpectedly (Kanzi was spending time close to the mother who was submitted to speech teaching experiments in Savage-Rumbaugh et al Labs in 1980s) started to display apparent language acquisition. However, even if this subject was able to produce structured utterances in response to requests or questions, or some sort of human behaviour designed to elicit an utterance, he was impaired in starting spontaneous vocal production and was able to produce just some words (Savage-Rumbaugh, Shankers, Taylor, 1998). Kanzi’s example, like many others, reported evidences that voluntary control is more highly developed manually than vocally in our closest primate relatives, supporting the hypothesis that language evolved from a system of manual gestures, with the gradual incorporation of vocalizations (Corballis, 2002; Gentilucci & Corballis, 2006).

This idea seems to be confirmed also by the evidence that humans still use hand and facial gestures when they speak.

Further support to gestural origins of language comes from the evidence that area F5 in the ventral premotor cortex of the monkey hosts a representation of both hand and mouth, and also at the single neuron level, these two effectors often coexist (Rizzolatti et al., 1988). In the same premotor cortical area mirror neurons were described (Gallese et al., 1996; Rizzolatti et al., 1996).

Note that the mirror system is actives both during observation and execution of the same motor act. It can thus be considered that the goal directed motor act wokrs in the mirror system as a sort of link between actor and observer. This is indeed similar to what occurs between sender and receiver of messages in communication. Rizzolatti and Arbib (1998) proposed that the mirror system was used as an initial communication system in language evolution. As Rizzolatti and Arbib (1998) recognized, the mirror system operates according to the same principles as postulated earlier by the motor theory of speech perception (Liberman et al., 1967), which holds that speech sounds are perceived in terms of how they are produced (in terms of movements or gestures of the articulatory organs), rather than as acoustic elements (Corballis 2010). In more recent versions of the theory (Galantucci et al 2006) the idea remains that the role of the speech motor system is not only to produce speech articulations but also to detect them.

Also MacNeilage (1998) gave a biological interpretation of language evolution focusing on the facial communicative primate expressions. Lip smacking, tongue smacks and teeth chatter is the result of repeated opening and closing movements of the mouth. This author hypothesized that spoken language evolved from this cyclical motion of the jaw involved even in older mammalian motor programs used for sucking, licking and chewing. Also human language is comparable in the opening/closing of the vocal tract during ingestive acts. When combined with vocal fold vibration this produces syllable-like utterances. According to MacNeilage one opening-closing cycle is called a frame. Motion of other articulators, such as tongue or lips, can be superimposed on these frames. This is the content. The combinations of these two behaviours produce syllables.

MacNeilage stressed the importance to focus on *motor act*. In order to understand speech evolution, it is necessary to understand the physical motor act on which it is based, and not just the mental mechanisms that underlie it. He suggested that the meeting point between frame and contents is Broca'a area, because this area likely derived from the sector of ventral premotor cortex controlling ingestive and communicative oro-facial behaviour.

MacNeilage (1998) proposals are controversial, but Rizzolatti and Arbib (1998) theory and the properties of mirror neurons are partially in agreement with it. Infact, discussing MacNeilage’s theory Rizzolatti and Arbib argued that the importance of Broca's area in speech evolution is for internally mapping the perceived hand movements made by others.

In line with Corballis (2003), Rizzolatti and Arbib (1998) argued that the first step of communication involved a brachio-manual communication system and then the system evolved including the oro-facial one. The consequent stage was the sound addition. The association of sounds to brachio-manual and oro-facial gestures assigned them a stronger referential meaning. If identical sounds were constantly used to indicate identical elements, a primitive vocabulary of meaningful sounds could have started to develop.

An important consequence of this new functional use of vocalization was the necessity of its skilful control.

When sound acquired a precise value and had to remain the same in identical conditions, this was most likely the cause of the emergence of human Broca’s area from an F5-like precursor that already had mirror properties (see BOX 1). The evolutionary pressures for more complex sound emission, and the anatomical possibility for it, were thus the elements that moved language from its brachio-manual origins to sound emission. Manual gestures progressively lost their importance, whereas, by contrast, vocalization acquired autonomy.

Arbib (2005a, 2005b) clarified that there was a stage in which the Mirror System moved from understanding transitive motor acts to the intransitive use of motor act for communication and defined this stage as "protosign".

As described in a previous section two categories of mirror neurons have been founded, that can support the proposal of a gestural origin of language: audio-visual (Kohler et al., 2002) and communicative (Ferrari et al. 2003) mirror neurons.

Other Language Evolution Theories

The most eminent linguist of our time, Noam Chomsky, asserts that human Language is something special. Starting from his first book Syntactic Structures, through the time he has sustained that true language is uniquely human and quite unlike communication among non-human animals.

According to this view, recent work on language evolution theory is presented by Hauser, Chomsky and Fitch, (2002). The authors explained how language could not be considered the result of gradual evolution or consequence shaping of pre-existing capability induced by natural selection. Comparing the animal communicative system to the human one, for the authors there is clearly an apparent discontinuity and human language is a new emerging property (Pinker and Jackendoff, 2004). Hauser et al underlie how the word 'language' has different meanings in different contexts and disciplines, so they delineated two conceptions of the faculty of language, one broader and another more restricted and narrow. Faculty of Language in a broad sense (FLB) included the sensory-motor and conceptual-intentional system whereas Faculty of Language in the narrow sense (FLN) consists in the abstract linguistic computational system. FLN is a component of FLB. It takes finite set of elements and recursively computes them in infinite arrays of discrete expressions, then passes them to the FLB, which processes and elaborates this information (comparing sound and meaning) in the use of language. The authors said that FLB is based on mechanisms shared with non-human animals while FLN is uniquely human. About the origin of this recursive ability Hauser and colleagues suggested that FLN evolved for reasons other than language but consequently to a genetic mutation it started computing his own information in a different way and interfacing with FLB inducing a more communicative elaboration of this latter.

The FLB-FLN theory was discussed by Jackendoff and Pinker (2005). They agreed with a non-monolith language analysis that permitted a combination of special language components with other general capacities in human or animal cognition. However they mainly criticized the lack of the adaptation processes like the evolution of a first symbolic communicative system.

Many other theories were formulated in order to explain language evolution. In particular it has been proposed that language could have been the consequence of a random change. Lieberman (1998) proposed that language itself evolved suddenly. This idea is sometimes called the "big bang" theory of language evolution and is attributed to Bickerton (1995) who described the "true language” as a “catastrophic event".

The discovery of the FOXP2 gene in the 1990s (this gene was discovered through molecular investigations of a family known as the KE family which suffered from severe speech and language deficits (Hurst et al 1990)) appeared to be the explanation of when language appeared. Some researchers (Enard 2002; Pinker 2003) suggest that examples like FOXP2 gene have to be considered a strengthen to the case of language being an adaptation. Andrews et al (2002) underline that the scientific demonstration that also our common Neanderthal ancestors had, like us, the FOXP2 gene could mean that they had language capabilities and suggests a sort of adaptation process that happened in human evolution at that point.

Biological Roots of Language

Human Language

Broca's Area

M. Dax studied more than 150 cases in which, without exception, a speech disorder was accompanied by a left hemispheric lesion. In 1836 he presented his data at a French medical conference. Those observations were long ignored because reported in a less important conference by a small town doctor. Twenty-five years later, the French physician Pierre Paul Broca, not aware of Dax description, was the first to prove beyond any doubt that there was a correlation between brain damage and mental disorders. He reported impairments in two patients; the first (Leborgne) was unable to produce any word or phrase. The second one (Lelong) exhibited reduced productive speech. Apparently, this lost ability to speak was not involving the intellectual capacity or the ability to understand others. After the patients’ death, Broca performed an autopsy of those brains discovering similar lesions in the left frontal lobe. The data were confirmed by brain examination performed on other patients with the same type of pathology. The cortical area of interest, namely the left inferior frontal cortex, has been known since then, as Broca's area and has been classified as the centre of language production. The deficit in language production described by Broca was classified as Broca’s aphasia.

Neuroanatomical studies defined Broca’s area in terms of the pars opercularis and pars triangularis of the inferior frontal gyrus (Aboitiz and Garcia 1997), represented respectively in Brodmann’s cytoarchitectonic map as areas 44 and 45.

Interestingly, it was observed that slow destruction of the Broca's area (for instance by brain tumours, traumatic events etc.) could leave speech relatively intact (Penfield and Roberts, 1959, Rasmussen and Milner, 1975). Furthermore human frontal opercular lesions have been often associated with oro-facial, laryngeal and tongue muscles paresis, while patients with lateral premotor lesions shown fingers exploratory movements impairment during objects manipulation.

Using different techniques, recent studies have shown that Broca's area is involved in several aspects of language. Electrical stimulation of the frontal lobe in awake patients produced marked interference with language output functions as well as language comprehension deficits (Schaffler, L. et al. 1993). Patients with lesions in Broca's area who exhibit agrammatic speech production also show inability to use syntactic information to determine the meaning of sentences (Caplan 2006). Also, a number of neuroimaging studies have implicated an involvement of Broca's area, particularly of the pars opercularis of the left inferior frontal gyrus, during the processing of complex sentences (Crewe et al 2005; Skipper et al 2007). Furthermore area 44 is significantly activated during the execution of distal movements, such as grasping and manipulation (Binkofski, et al. 1999; Gerardin, et al. 2000; Fadiga and Craighero 2006). This area is not just related to execution but it activates also during the motor imagery (Gerardin, et al. 2000; Lotze and Halsband 2006) and the observation of meaningful hand motor acts (Fadiga et al 1995; Buccino et al 2001; Binkofski and Buccino 2006).

All these findings give us the possibility to conclude that Broca's area, and particularly Brodmann’s area 44, classically considered mainly involved in the motor control of oro-pharyngeal movements and in language production, is rather a sensorimotor interface able to integrate sensory aspects of the stimuli and cognitive tasks with their own motor representation of motor acts involving hands and face-mouth. Broca's area seems to be involved in higher-level systems that originally thought.

Wernicke’s area.

Since the late 19th century, Wernicke's area has been considered the posterior region of the cerebral cortex related to language. In 1874, Karl Wernicke, a German neurologist and psychiatrist, suggested, based on ten clinical cases, that in the left temporal lobe (the posterior section of the superior temporal gyrus), caudally to primary auditory cortex there was an area implicated in language control (Pinel, 2000). This area encircles the auditory cortex on the Sylvian fissure (part of the brain where the temporal lobe and parietal lobe meet) and is neuroanatomically described as the posterior part of Brodmann’s area 22. Wernicke believed that this area was involved in language comprehension and hypothesized that its selective damage would result in a form of receptive aphasia characterized by poor spoken and written language understanding and by a senseless language production while speech retained a natural-sounding rhythm and a relatively normal syntax. As a result of that, language was largely meaningless. That hypothetical form of aphasia was called "Wernicke's aphasia". He was also the first to distinguish different forms of aphasia in an anatomical framework. Wernicke proposed the existence of a large bundle of nerve fibers connections (subsequently named arcuate fascicles) between the two known speech systems, the first related to speech production and located on the left frontal cortex (Broca's area) and the second constituted by Wernicke's area. His idea was that a disconnection between the motor and sensory systems of language would lead to a unique impairment distinct from both Broca's and Wernicke's aphasias, which he termed *Leitungsaphasie* (Conduction aphasia).

Since then, Wernicke’s observations have been confirmed several times. Neuroscientists now agree that there is a loop around the lateral sulcus (Fissure of Sylvius) in the left hemisphere of the brain involved both in understanding and producing spoken language, but new neuroimaging techniques show that Wernicke's predictions were not totally true (Mayeux and Kandel, 1994). In fact, as anticipated in the section of Broca's area, language comprehension doesn’t seem to be an exclusive property of Wernicke's area. Even when patients with Wernicke's area lesions have comprehension deficits, these impairments are usually not exclusively restricted to language processing. For example, one study found that patients with posterior lesions had trouble also in understanding non verbal sounds like animal and machine noises (Saygin et al 2003). Some people may use the right hemisphere for language, and isolated damage of Wernicke's area (sparing white matter and other areas) may not cause severe Wernicke's aphasia (Bogen et al 1976; Dronkers et al 2000).

New research demonstrates that the arcuate fasciculus, previously thought to connect Wernicke's and Broca's areas, connects, instead, posterior language areas to premotor/motor areas (Bernal and Ardila 2009). But it's true that the language loop anticipated by Wernicke is founded in the left hemisphere in about 90% of right-handed persons and 70% of left-handed persons. Recent researches underline that language is one of the functions performed asymmetrically in the brain. In humans, the planum temporale is predominantly larger in the left hemisphere, especially among right handed individuals (Galaburda et al. 1978; Hepper et al. 1991; Naidich et al. 2001).

However, the uniqueness of human brain asymmetry has been challenged by discoveries of behavioral and neuroanatomical asymmetries in other species (Rogers & Andrew 2002). In particular, gross structural asymmetries have been observed in non-human primates, including chimpanzees, for region likely homologues of the areas implicated in human language and speech production (e.g. Gannon et al. 1998; Hopkins and Cantalupo, 2003).

Based on modern knowledge concerning human Wernicke's and Broca's areas and their homologues with our closest living relatives, we can speculate that these areas served some pre-adaptive functions in non-human primates by conveying information about conspecific communication to the motor act planning system, which was later co-opted in human evolution to subserve language.

Monkey vocalization

Primates can use vocalization for communicative messages among conspecifics in various contexts such as avoiding predators, defending against aggressors, traveling as a group and discovering food. According to Jürgens and Ploog (1970), vocalizations were consequences of changes in the internal state induced by an external or internal stimulus. In compliance with Simonyan and Jürgens (2002) vocalizations are almost entirely genetically determined. Considering monkey calls just as mean of emotional communication leads us to assume that subcortical areas could be sufficient to carry out their neural control. However, various behavioural evidences, as illustrated in previous section, has demonstrated that monkey vocalizations can convey a more complex information than the sole emotional state, suggesting a possible cortical involvement of cortical structure in monkey vocalization. During the last four decades several electrophysiological and anatomical studies were carried out to build a general model for voice control and the results show that also in non-human primates voice production needs joint efforts between subcortical and cortical areas.

Vocalization is a complex behaviour pattern, made up of essentially three components: respiratory movements, laryngeal activity, and supralaryngeal (articulatory) activity.

The motoneurons involved in vocal control (neurons controlling the vocal folds, the respiratory muscles and the shaping of lips, tongue and jaw movements), are distributed along the neuraxis from the level of the pontine brainstem down to the lumbar spinal cord (Jürgens 2002; Yoshida et al 1992). The production of specific vocal pattern requires that the activity of phonatory motoneuronal groups be coordinated.

Jürgens and Ploog (1981, Jürgens 2002, 2009).) proposed a hierarchically organized circuit (limbic cingulo-periaqueductal pathway) at the base of vocal control. The model was composed of four levels:

1) at the first level there is the anterior limbic cortex involved in the voluntary aspect of vocal production. The researches that classically allowed to identify the anterior cingulate cortex (ACC), as an important node in the neural network underlying the non-human vocal production, were primarily lesions studies. In Sutton, Larson and Lindeman's (1974) study, some rhesus monkeys as been trained to emit a relatively prolonged call. At the end of the operant conditioning task the anterior cingulate/subcallosal gyrus was bilaterally removed,leading to the loss of phonatory performance. Similar results were observed by Aitken (1981). Few years later, West and Larson (1995) recorded single-neurons activity in this cortical region during monkey performance of self-paced vocalizations. The authors identified voice related cells displaying an anticipatory discharge with respect to sound emission. The results of this study confirmed the presence of a vocal cingulate region (Brodmann's area 24, 25 and part of 32) supporting Jürgens and Ploog (1970) data.

2) At the second level of the model there are subcortical limbic nuclei, such as the amygdala, (Jürgens, 1982), related to emotional vocalizations.

3) At the third level, there is the periaqueductal gray matter (PAG) and the adjacent tegmentum, considered to act as a bridge between the output of the limbic system to the sensory and motor nuclei (forth level of this hierarchy). The

involvement of PAG in monkey vocal production has been suggested from several studies. Lesion studies of this region have shown both temporary and permanent mutism in monkeys (Jürgens and Pratt, 1979a ;Jürgens and Ploog, 1981), but also in other species, such as cats (Adametz et al 1959), rats (Churand et al 1972) and also in humans (Esposito et al 1999). Electrical stimulation of PAG inducing vocalization in primates was first observed in chimpanzees (Brown 1915). Subsequently the same results were obtained with midbrain stimulation in rhesus monkeys (Magoun and Atlas 1937), squirrel monkeys (Jürgens and Ploog 1970; Jürgens, 1979; Larson and Kistler, 1984), gibbons (Apfelbch 1972) and humans (Sem-Jacobsen and Torkildsen 1960). The direct control by PAG of vocalization in primates has been suggested observing the short latency (around 12 ms) between this region electrical stimulation, the consequent EMG laryngeal muscle activity and the subsequent onset of vocal production (Ortega et al 1988). The direct control interpretation was also supported by single-unit recording studies carried out in this region of rhesus and squirrel monkeys (Larson 1991; Düsterhöft et al. 2004). Durig the electrophysiolocal experiments numerous vocal-related neurons were recorded and classified into 4 type: (a) cells showing an increased activity immediately before, but not during vocalization; (b) cells showing an increased activity immediately before and during vocalization; (c) cells increasing their activity at vocalization onset or shortly after it; (d) cells decreasing their activity during vocalization (Larson 1991; Düsterhöft et al. 2004).

4) The fourth and final level, is constituted of the sensory and motor nuclei (mainly belonging to the reticular formation), responsible of the actual execution of sound production. Single cell recordings demonstrated vocalization-related neurons in the squirrel monkey reticular formation (Jürgens 2002; Lüthe et al 2000) and brain stimulation and lesioning studies clearly showe that the reticular formation plays an important role in vocal production (Jürgens et al 1986; Kirzinger and Jürgens 1985).

Anatomical studies confirmed Jürgens and Ploog model (1981). Cortical projection from the cingulate cortex to the PAG and then from PAG to reticular formation of the lower brainstem were observed from Muller-Preuss and Jürgens (1976), Manthyn (1983) and Larson (1991) studies.

Also lesions studies corroborated the hierarchical model. The destruction of the

PAG abolishes vocalizations elicited from the anterior cingulate cortex, but does not abolish vocalizations elicited from the reticular formation (Jürgens and Praat 1979b; Siebert and Jürgens 2003); destruction of the anterior cingulate cortex leaves vocalizations elicited from the PAG and reticular formation unaffected (Kirzinger and Jürgens 1985; Jürgens and Praat 1979).

Apart from limbic cingulo-periaqueductal pathway, controlling the readiness to vocalize, there is a possible second pathway, responsible for the patterning of vocal utterances and was found in the reticular formation of the lower brainstem (Jürgens 2009). The highest level of this system is represented by the motor cortex.

More specifically it is represented by the most lateral part of the premotor cortex where lips, jaw tongue and larynx movements are represented. Of particular interest for vocal production is the area of larynx representation. When this region is electrically stimulated, bilateral vocal fold adduction is obtained as observed in the same period by Hast and colleagues and Jürgens (Hast et al., 1974; Jürgens and Ploog, 1970).

To specify the course of this second pathway, Symonian and Jürgens (2003) injected anterograde tracers in squirrel monkey cortical larynx area finding direct projections with more subcortical regions (putamen, caudate nucleus, and claustrum, to various thalamic nuclei, such as the ventrolateral, ventral posteromedial, mediodorsal, central lateral, and pulvinar nuclei, to the pontine gray as well as to the solitary tract nucleus, spinal trigeminal nucleus, and reticular formation of the lower brainstem) but no one was observed through motor cortex and nucleus ambiguous (where laryngeal motor neurons are located). In order to identify which monkey brain structures represent the connection between motor cortex and laryngeal motor neurons a combined stimulation and inactivation study was recently performed by Jürgens and Ehnreich (2007). Results showed that the only area capable of blocking cortically induced vocal fold movements, on both sides, is the reticular formation of the dorsocaudal medulla oblongata.

Concerning human vocal production, neurological literature reveals that speech disorders do not only occur after lesions in the motor cortex and reticular formation, but also after lesions in the putamen, ventrolateral thalamus, and pontine gray (structures receiving a direct input from the motorcortical larynx area) (Jürgens 2002).

The author suggested a model to explain these findings (Figure 4).

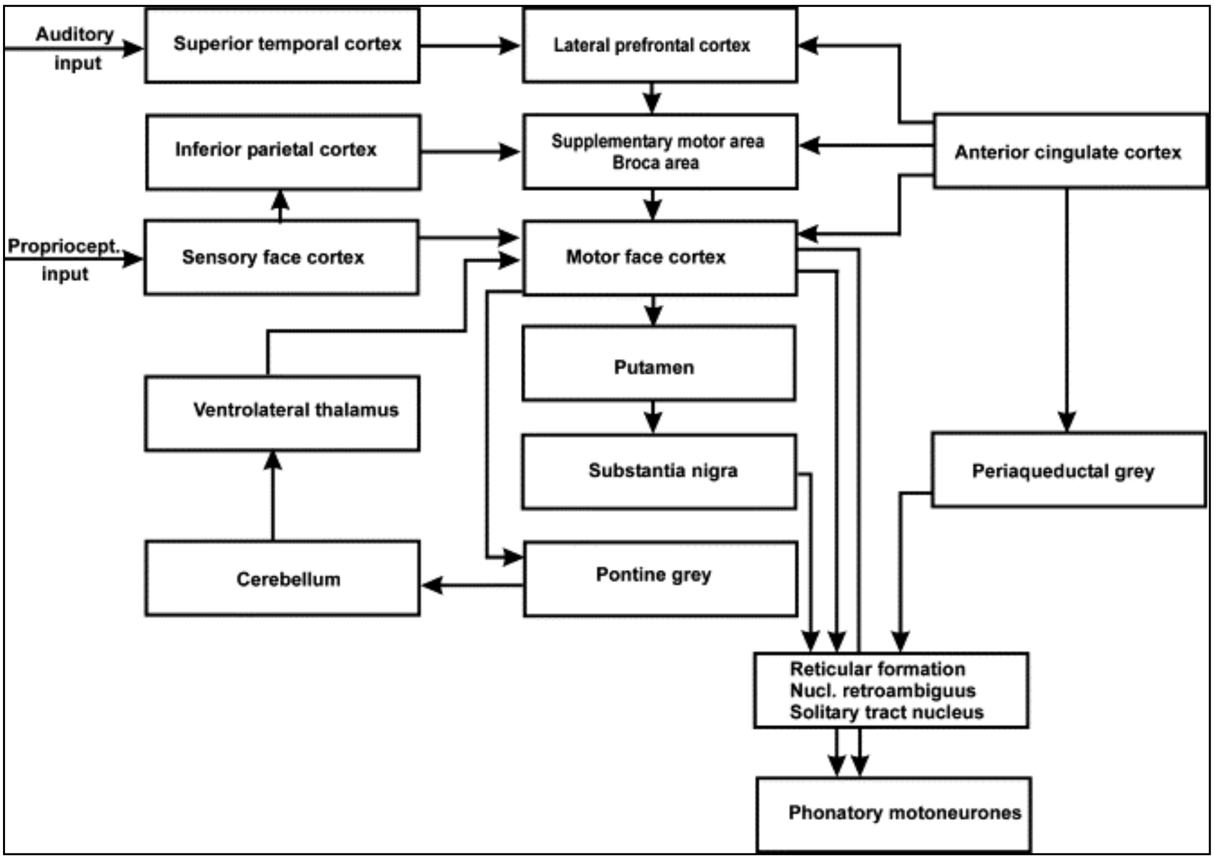

Figure 4. Scheme illustrating the anatomical relationships among various structures involved in vocal utterances control (Jürgens 2002).

This model assumes that motor commands sent by the motor cortex to the motor neurons via the reticular formation, are pre-processed by structures whose lesion leads to an impairment of speech control. The motor cortex is known to project to the pontine gray, the pontine gray projects to the cerebellum, and the cerebellum projects back to the motor cortex via the ventrolateral thalamus. As mentioned earlier, all these structures cause voice disorders, when damaged bilaterally. A second loop, runs from the motor cortex to the putamen, from there to the globus pallidus, and back to the motor cortex again via the ventrolateral thalamus. In this model, putamen, globus pallidus, ventrolateral thalamus, pontine gray, and cerebellum would provide the motor cortex with information necessary to produce the correct motor commands.

In this model, it is considered also the limbic vocal control pathway, which, differently from that originating the motor cortex, is shared between human and non-human primates. In the Jürgens' s model of vocal control, the main element is considered to be the reticular formation where both pathways converge to allow any kind of vocal emission.

Aims of This Work

Aims of this work

As described in the previous chapters, non-human primates vocalize in a wide range of contexts and possess a repertoire of vocalizations used to designate objects, events or affective states (Hauser et al., 2002; Seyfarth and Cheney, 2003). Several species of monkeys are capable of modifying their vocalizations according to environmental parameters (Sinnott et al., 1975; Brumm et al., 2004; Egnor and Hauser, 2006). However, no monkey and ape species are granted with enough flexibility to learn completely new vocal patterns (see Yamaguchi and Izumi, 2008).

The lack of flexibility in the vocal patterns is likely due to a limited capacity in the voluntary control of the vocal apparatus. Although behavioral studies have demonstrated that macaque monkeys can achieve a significant level of voluntary vocal control when submitted to operant conditioning tasks (Sutton et al., 1973; Aitken and Wilson, 1979; Hihara et al., 2003; Yamaguchi and Izumi, 2008), the success rate of vocal training remains variable (Yamaguchi and Myers, 1972; Pierce, 1985).

It has been hypothesized that vocal potential of non-human primates is impaired by the high position of the larynx relative to the skull base. However, in several mammalian species, including non-human primates, the larynx has been shown to have more movement flexibility than classically assumed (see Fitch, 2000). Consequently, the reasons for poor voluntary vocal control probably rely more on limitations of the neural machinery.

As decribed above, the neural underpinnings of vocal production in nonhuman primates have been classically attributed to the brainstem and to areas of the mesial cortex (West and Larson, 1995; Jürgens, 2002). However, a potential candidate for voluntary control of vocalization is the ventral premotor cortex (PMv). This region is well known to control hand and mouth actions (Rizzolatti et al., 2004) and contains a larynx movements representation, as shown by electrical stimulation studies (Hast et al., 1974; Jürgens, 2002). Furthermore, anatomo-functional data support a homology between the anterior half of PMv (F5) of macaques and part of human Broca’s area 44 (Rizzolatti and Arbib 1998; Fogassi and Ferrari 2007) which is involved in speech production (Broca, 1861; Penfield and Rasmussen, 1950; Rasmussen and Milner, 1975; Ojemann et al., 1989) and larynx control (Brown et al., 2008). Single neuron and brain imaging studies indicate that monkey PMv has properties suitable to be exploited for communication (Kohler et al 2002; Ferrari et al 2003; Gil-da-Costa 2006). However, these studies are not conclusive in clarifying the controversial issue of whether human speech evolved from newly-formed area or from a monkey precursor. Although monkey PMv has been considered to have a minor involvement in vocalization (Sutton et al., 1974; Aitken, 1981; Jürgens, 2002), the properties of its neurons in this function have not been directly investigated.

The aim of this study was two fold: first to assess whether it is possible to train macaque to vocalize frequently enough to allow for an electrophysiological recording of neuronal properties during vocalization, second to verify whether neurons in macaque PMv are directly involved in the control of conditioned vocalization. To this aim we trained two macaque monkeys to produce conditioned vocalization by means of an operant conditioning paradigm, and then we recorded the activity of single neurons in the lateral sector of PMv (area F5) during voluntary-controlled vocalizations. Finally, we verified by electrical microstimulation whether the recorded regions has a direct motor output on the mouth and larynx.

Materials and Methods

Vocalization Training

Two captive-born and individually housed adult pigtailed macaques (*Macaca nemestrina*) served as subjects. All experimental protocols were approved by the Veterinarian Animal Care and Use Committee of the University of Parma as well as by the Italian Ministry of Health. All experiments complied with the European law on the humane care and use of laboratory animals.

Both monkeys were chosen because they displayed a higher level of spontaneous coo-call emission with respect to their peers housed in the laboratory. Before starting the training, a baseline level of spontaneous coo-call (frequency of calls/session) was assessed. Before starting the experimental training, monkeys were gradually habituated to the presence of the experimenter and to the experimental setting. The monkeys become use to be guided out of their cages by means of a leash, to gradually approach and sit in a primate chair under conditions of partial restraint. During the habituation period, food (fruits and vegetables) and liquid (water and fruit juice) were used as a reward.

The monkeys were then submitted to an operant conditioning task designed to increase their vocalization rate.

The task initially consisted of a shaping procedure, lasting 5 days, in which the monkeys were rewarded by the experimenter with a piece of palatable food any time they emitted a coo-call. At the end of this procedure, the following structured task was introduced:

(a) The monkeys were facing a small table (57,7 cm long, 13,8 cm wide and 1 cm thick) and had to emit a coo-call only when a piece of food (the size of which was gradually reduced in order to enlarge the daily training time), was put on the table by the experimenter, out of reach.

(b) The monkeys had to emit a vocalization within 30 seconds of presence of food on the table (“Food” condition) in order to get it as a reward; if they did not vocalize after 30 sec., food was removed from the table. This time period was chosen to ensure that the monkeys vocalizations were associated to the presentation of food and not to other events.

(c) At the end of the “Food” condition period, a “No food” condition (30 sec. duration) followed, where no food was present on the table and no reward was delivered to the monkeys, even in the case they vocalized. The "No Food" condition was introduced to reinforce the association between the absence of a stimulus and the no-coo production (see Fig. 5). Each session of the task was 30 minutes long. Since the task was very sensitive to monkey fatigue, a maximum of two sessions/day were carried out.

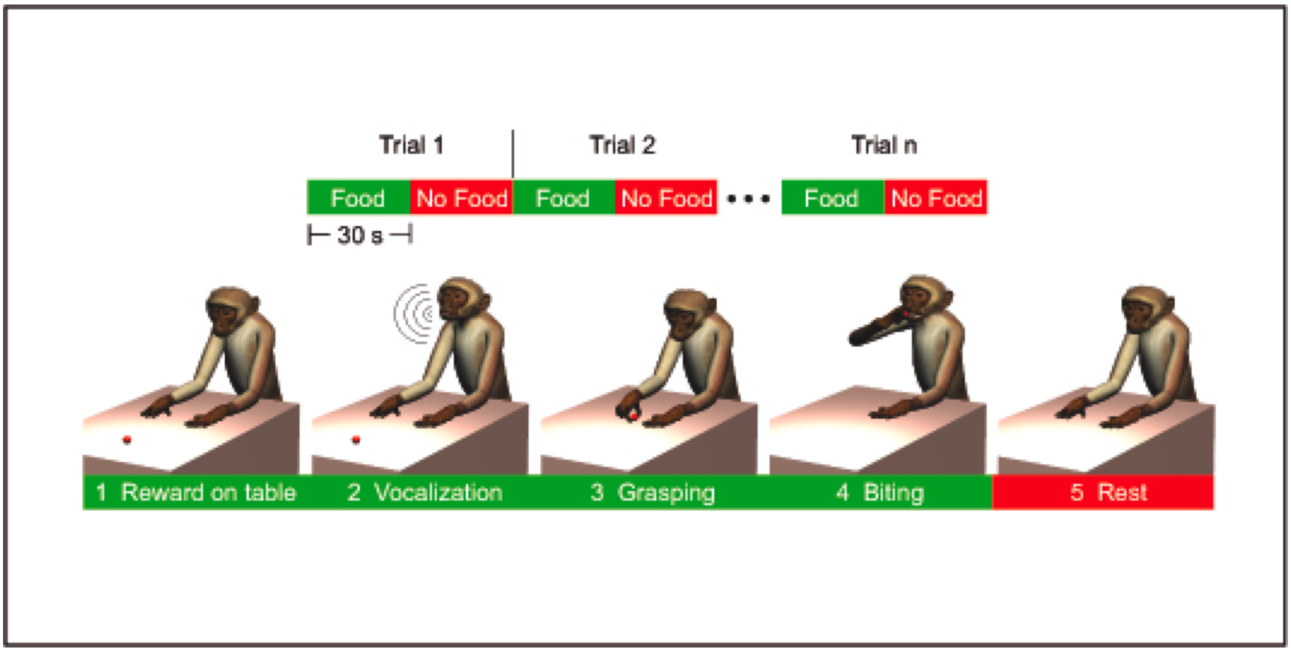

Figure 5. Vocal operant conditioning task. *Top.* Each trial consisted in a "Food" (green) and a "No Food" (red) condition. *Bottom.* Schematic illustration of a successful task trial. In the "Food" condition the food was presented on a table (1); if the monkey emitted a coo (2), the food was given to it. The monkey grasped the food (3) and brought it to the mouth (4). In the "No food" condition the food was not present and the monkey was not required to vocalize (5).

The task was performed in three subsequent phases with the purpose of gradually training the monkey to perform the task during the electrophysiological recording. Each new phase involved a change in the environmental conditions. In the first phase, the task was performed when the monkey was in its home cage (Stage I). In the second one, the monkey performed the task while seated in the primate chair with the head free (laboratory training, head free – Stage II). In the third phase, the task was performed while the monkey was seated on the primate chair with the head fixed by a head holder designed for electrophysiological recordings (laboratory training with the head fixed – Stage III). During the task, other types of vocalization like grunts or shrieks were never reinforced. Throughout the whole training, the behaviour of the monkeys was carefully recorded on a data sheet, especially the frequency of vocalizations produced. We also recorded a specific orofacial configuration that normally accompanies coo-call execution, but that is performed without sound production. This behaviour was observed in a previous lesion study (Aitken, 1981) and described as “silent vocalization” (SV) because the facial display is very similar to that occurring during vocalization.

Surgical procedure

As vocalization training with the head free was deemed complete, each monkey underwent surgery. The surgical procedures for implanting head restraining system and recording chamber (Fogassi et al., 1996; Rozzi et al., 2006) was conducted under general anesthesia and in aseptic conditions. Each animal was deeply anaesthetized with ketamine hydrochloride (5 mg/kg i.m.) and medetomidine hydrochloride (0.1 mg/kg i.m.) and its heart rate, temperature and respiration were carefully monitored and kept within physiological range. The head implant included a head holder constituted by four hollow metal cylinders used to hold each one a cylindrical bar and a custom-made titanium chamber to allow single-unit recording. The cylinders were placed bilaterally in the anterior temporal and occipital regions. The chamber was placed in order to include area F5.

The monkeys were placed in a stereotaxic apparatus, an incision was made in the scalp, and the skull was drilled to remove the bone overlying the target region. Then, the implanted elements were anchored with titanium MF cortex screws self-tapp (∅1,5mm) and subsequently firmly stacked to the skull through antibiotic acrylic-methacrylate (Antibiotic Simplex ©).

To construct a correct grid of the recording penetrations at the end of surgical implantation, an external landmark was calculated, referred to an external stereotactic zero and related to one of the corners of the chamber.

Electrophysiological recording and microstimulation

During the recording sessions, the awake monkeys were seated on a primate chair with the head fixed by the head holder.

Neuronal recording was performed using an Alpha Omega electrode device (Alpha Omega Eng., Nazareth, Israel) that was attached to the head holder. Neurons were recorded using tungsten microelectrodes (impedance 0.5–1.0 MΩ, measured at 1 kHz) held by a micromanipulator fixed to the head-stage. The electrodes were lowered by an electric engine, controlled by a dedicated software package (EPS; Alpha Omega), with an angle of 35° with respect to the vertical axes in order to penetrate perpendicularly to the cortex through the intact dura. The definitions of the recording parameters were performed by a MCP-Plus (Alpha Omega) software. Neuronal activity was amplified, monitored on an oscilloscope and played through an audio amplifier and sent to a PC where it was displayed online and saved for the offline analysis. When possible, individual action potentials were isolated with a dual voltage-time window discriminator (Bak Electronics, Germantown MD, USA). In order to align the neuronal activity with the external events, the experimenter manually closed a contact-detecting circuit at the onset of each monkey vocalization. The output signal from the voltage-time discriminator was monitored and fed to a PC for analysis. All recording sessions were video-recorded.

Intracortical microstimulation was also carried out with the same microelectrodes used for recordings (train duration: 50 ms; pulse duration: 0.2 ms; frequency: 330 Hz; current intensity: 3-40 μA). When recording sites showed neuronal activity related to vocalization, longer stimulation trains were used (train duration: 500-1000 ms; pulse duration: 0.2 ms; frequency: 50-200 Hz; current intensity: 30-60 μA). For each penetration site, stimulation trains were first applied at the maximum current intensity at least four times. If any overt movement was detected visually or by touch in at least half of the stimulation trials plus one, the site was considered excitable. Stimulations at lower current intensity were also made to find stimulation threshold. The lowest current at which a movement was detected for at least half of the trials plus one was considered the stimulation threshold.

Neuronal testing and functional mapping

In the two animals, the size of the recording chamber in its rostro-caudal and medio-lateral axes (about 20 mm × 25 mm) was such as to allow recording from the whole ventral premotor cortex, including also area F1 (primary motor cortex) and the caudal part of the frontal eye fields (FEF) in order to define the premotor and prefrontal cortex borders.

After chamber implantation, the ventral part of the agranular frontal cortex was functionally explored (by means of single neuron recordings and intracortical microstimulation) in order to assess the location of areas F1 (primary motor cortex), F4 and F5 (ventral premotor cortex) and to identify the sector of F5 where neurons related to mouth actions are mostly located. The recorded area was sampled with a 1 mm grid (Figure6).

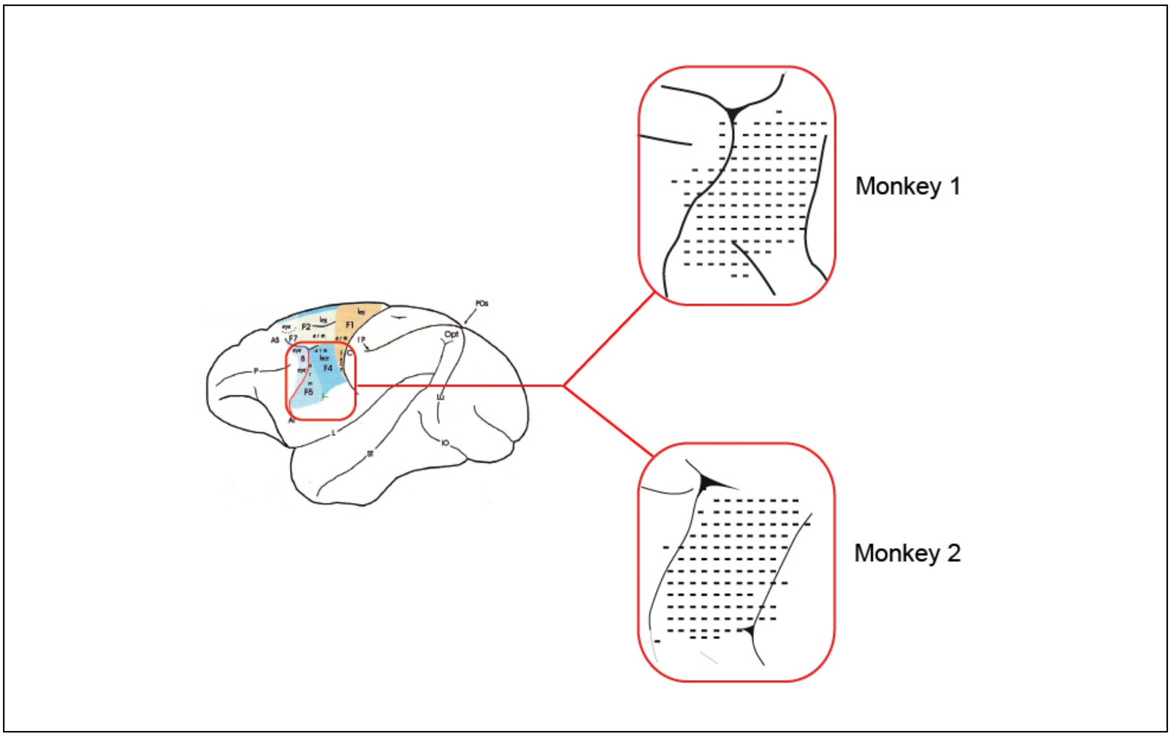

Figure 6. *Left*. Lateral view of macacque monkey left hemispheres with red-circled area of interest. *Right*. Cortical regions, of Monkey1 and Monkey2 respectively, functionally explored to assess the location of areas F1, F4 and F5. Each segment indicate the location of electrode penetration.

The criteria used to functionally characterize the different areas were the following Area F1: low threshold of excitability to microstimulation (typically 3-5 μAs when stimulating deep layers), vigorous discharge during active movements, response to passive somatosensory stimuli virtually from all recorded sites. Area F4: moving the electrode rostrally from F1 hand field, elicitation of proximal and axial movements with electrical stimulation, increase in stimulation threshold, appearance of visual responses, presence of large tactile receptive fields located on the face and body, and of visual peripersonal receptive fields over the tactile ones. Area F5: going further rostrally, reappearance of distal hand movements requiring higher stimulation currents than F1, presence of a large number of neurons discharging in association with goal-oriented hand and mouth movements, visual responses to the presentation of 3D objects or to observation of complex actions. The identification of area F5 on the basis of its functional properties has been histologically confirmed and validated by several previous experiments (Rizzolatti et al. 1988, Rizzolatti et al. 1996, Gallese et al. 1996a and Fogassi et al. 2001, Rozzi et al. 2006).

During each experimental session, the electrode was inserted through the dura until the first neuronal activity was detected. The spontaneous activity was then carefully observed and correlated to the monkey behaviour. The electrode was then deepened into the cortex in steps of 250 μm until the border between the gray and the white matter was reached. At each site, multiunit and single-unit activities were recorded and their correlation with any type of sensory stimulation, motor activity and monkey vocal production were noted on a protocol and subsequently inserted into a database.

In a following experimental phase, the recording sessions were concentrated in the regions in which mouth and vocalization related neurons were found.

A total of 205 electrode penetrations were carried out in the lateral parts of areas F4 and F5 of the two monkeys. Overall, during this preliminary mapping, vocalization-related neurons were found in 7 electrode penetrations, all of them located in the rostral half of the recorded region. Sectors in which activity related to vocalization was found were subsequently more densely sampled.

The neurons were first "clinically" tested in order to evaluate their motor, somatosensory, visual or auditory properties. If motor properties related to mouth were found, the vocalization task described above started, otherwise the electrode was slowly lowered in steps of 250 μm. When a single neuron activity was isolated, we tested the unit for vocalization as well as for other behaviours related to mouth/throat movements (e.g. mouth grasping, licking, swallowing and chewing. The multiunit activity was also recorded continuously for subsequent offline analysis.

Besides clinical testing, auditory properties have been investigated more in depth in order to evaluate whether vocalization-related neurons could be also activated when the monkeys were listening at vocalizations emitted by conspecifics or by themselves (mirror responses). To this purpose, at each recording steps we played back the vocal emissions of the tested monkeys and other vocalizations recorded from several monkeys of our facilities. The vocalizations used for the mirror responses test were spontaneous and conditioned coo-calls, monkey screams and grunts. In order to remove hissing and subsonic rumble these were previously filtered. Furthermore, in order to rule out the possibility that the neuronal discharge during vocalization was due to simple auditory feedback, we recorded on-line the monkey vocalization and immediately played back the same vocalization that was effective during monkey sound emission. Reversed (in time domain) playbacks were also presented to the monkeys. Those sounds were still equipped with the same acoustic properties and frequency profiles (preservation of the spectral contents) of classical vocalizations, but were hypothetically meaningless for the animals.

The same software (Cool Edit/Audition, Adobe) used for recording vocalization was employed for sounds playback. All acoustic stimuli were presented using a single high-quality digital loudspeaker (Genelec S30D) having a frequency response reaching 48 kHz (with an amplitude variation within ± 2.5 dB range), placed 2 m from of the monkey.

Off-line data analysis of neuronal trace

For each recorded site, the time occurrence of behavioural events of interest (e.g. vocalization, biting, licking, mouthing, hand grasping, etc.) was precisely identified by means of the video recordings synchronized with neuronal activity trace. The neuronal activity trace associated with the various behaviours was submitted to a spike sorting analysis performed with a Matlab©-based program (wave_clus, Caltech). When possible, we acquired the activity of each neuron for several trials during the monkey performance of different behaviours.

For each behaviour, neuronal discharge was aligned with specific events: (a) vocalization, with the onset of sound emission; (b) mouth grasping, with the contact between mouth and food; (c) licking, with the contact between tongue and food, (d) chewing, with the first maximal aperture of the mouth, (e) hand grasping, with the contact between the hand and the food. This procedure allowed to construct rasters and PSTH (Peristimulus Time Histograms or histograms of the times at which neurons fire to visualize the rate and timing of neuronal spike discharges in relation to an external stimulus) and to subsequently compare the neuronal discharge recorded during monkey performance of different behaviours.

Considering the discharge of two epochs having duration of 400 ms each, the analysis of neuronal activity was carried out for each behaviour. The choice of this timing is based on an analysis revealing that the duration of the relevant behaviours is within a range of 350-450 ms. The epochs were identified according to criteria that were adapted to the specific behavioural features. *Vocalization*. Epoch 1: from the beginning of lips opening before vocalization to sound emission onset; epoch 2: from sound emission onset to the end of lips protrusion. *Licking, mouth grasping and chewing*. Epoch 1: from beginning of mouth opening to contact with food; epoch 2: from contact with food to mouth closure. *Hand grasping*. Epoch 1: from beginning of hand opening to contact with food; epoch 2: from contact with food to the complete hand closure. For the baseline condition (rest), we considered an 800 ms period during which the monkeys were not performing any mouth or hand movement. In each trial, the mean discharge frequency was calculated for each 400 ms epoch.

Neuronal classification.

The categorization of neurons as vocalization-selective or vocalization-related was based on the mean firing rate comparison across conditions (biting, licking, behaviour, etc) and epochs (epoch 1 and 2) by means of ANOVA and Newman–Keuls posthoc tests. Neurons were categorized as vocalization-related or vocalization-selective according to two criteria. Neurons having a mean firing rate significantly higher for vocalization that for the rest condition were classified as vocalization-related (criterion 1). Neurons satisfying criterion 1, but also showing a mean firing rate significantly higher for vocalization than for any other condition were classified as vocalization-selective (criterion 2).

Voice analysis

During the electrophysiological recording sessions, happened that monkeys vocalized in the 30 sec. period in which no food was presented on the table. Acoustically these sounds were not much different from the coo calls reinforced by the experimenters. Despite this acoustic similarity, no neural discharge was associated with the unrewarded vocal productions.

Like human voice, monkey vocalizations consist of sounds produced by contraction of the vocal folds. Each sound is a mechanical wave composed of different frequencies and transmitted through the air. Any process that quantifies the various amounts (e.g. amplitudes, powers, intensities, or phases), versus frequency can be called spectrum analysis. The frequency spectrum can be analyzed via a Fourier transform of the signal. The spectral peaks of the sound spectrum of the voice is defined Formant. Formants are the distinguishing or meaningful frequency components of voice.

The formant with the lowest frequency is called Fundamental (F_0) and is directly associated to vocal cords vibration. The other formants with increasing frequency value are called the First (F_1), the Second (F_2), and the Third (F_3).

Most often the two first formants, F_1 and F_2, are enough to disambiguate the vowel. These two formants determine the quality of vowels in terms of the open/close and front/back dimensions (which have traditionally been associated with the position of the tongue).

To assess whether differences or similarities in the acoustically profile of vocalizations emitted in a “Food” and “No Food” period were present and also to understand why the neural discharge was present only during the food-vocalizations production, the formants of both kinds of vocalizing sounds were visualized by spectrograms and a spectrogram analysis was then carried out.

Statistical analysis

Behavioural and single neuron analysis.

For the behavioural analysis, the monkey performance was calculated in terms of number of trials/session in which at least one vocalization or SV was emitted during the “Food” or “No food” condition. The Wilcoxon matched pairs test was used to assess for significant difference in the frequency of vocalization between training phases and between conditions (“Food” or “No food”). The neurophysiological data on single neurons were analysed with a two-way ANOVA for repeated measures (alpha: $p < 0.05$) (factors: Epoch and Condition) followed by a Newman–Keuls test (two-tail, $p < 0.05$).

PMv raw activity measure and population analysis.

In order to compare neuronal activity associated with the emission of conditioned (“Food”) and spontaneous calls (“No food”), we performed a population analysis, by applying a method that calculates neuronal activity using the RMS estimate of the raw multi-unit activity (Moran et al., 2006).

The RMS estimate is defined as follows:

$$RMS(\vec{X}) = \sqrt{\frac{\sum_{i=1}^{n} X_i^2}{n}}$$

Where $(\vec{X})$ is the vector of the analog signal trace, X_i is each sample squared, and n is the number of samples. Since RMS is influenced by electrode properties, amplification gain, or other factors, the RMS was normalized (NRMS) on the baseline activity recorded when the monkey did not produce any movement (baseline NRMS activity = 1). The bin width of the RMS is 20 ms, the same as in single-unit PSTH analysis.

Normalized RMS data were submitted to an arcsin-square root transformation. A 2 X 2 repeated measure ANOVA (Factors: Condition and Epoch) has been applied to the NRMS for “Food” and “No Food” conditions in the two epochs before (Epoch 1) and during (Epoch 2) the vocalizations.

Voice recordings and analysis

All monkey sounds emitted during the experiments were recorded by means of a high definition microphone (Earthworks TC30) placed near the monkey. They were digitalized at 16 bits quantization and at 44.1 kHz sampling frequency through an analog/digital interface (Mindprint AN/DI pro) and sent to a PC where they were saved for offline analysis. Each vocalization was trimmed from other sounds and band-pass filtered from 30 Hz to 10 kHz by means of a sound editing software (Cool Edit/Audition, Adobe) in order to remove hissing and subsonic rumble. All voice analysis was performed with Praat analysis software package (GSU Tool). The differences in acoustic measures between “Food” and “No food” conditions were analyzed with paired t-test (two-tail, $p < 0.05$).

Histological procedures and data analysis

About 10 days before sacrificing the animals, electrolytic lesions (10 μA cathodic pulses for 10 s) were performed at known coordinates at the external borders of the recorded regions. Each animal was then anaesthetized and transcardially perfused as previously described (Rozzi et al., 2006). The brain was frozen and cut in 60 μm-thick sections, with each second and fifth section of a series of five stained using the Nissl method (thionin, 0.1% in 0.1M acetate buffer pH 3.7). The locations of penetrations were then reconstructed on the basis of electrolytic lesions, stereotaxic coordinates, depths of penetrations and functional properties.

Results

Behavioural performance in vocalization

The vocal operant conditioning task consisted of 115 sessions in Monkey 1 and of 109 sessions in Monkey 2. The overall progress of the two monkeys performance throughout the various phases of training is shown in Figure 7.

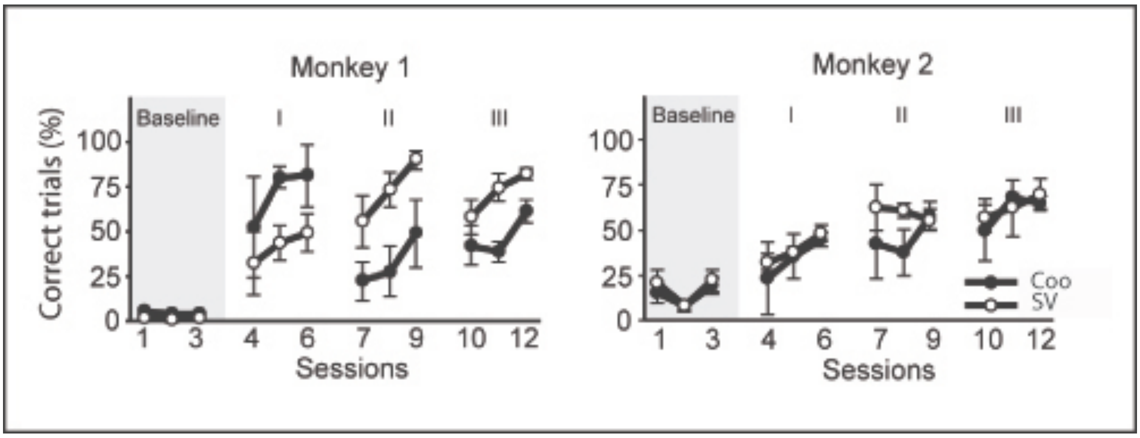

Figure 7. Monkeys performance throughout baseline and training phases (I, II and III, see methods). Each dot of the line graph represents the average percentage of the performance on three consecutive training sessions. For the sake of clarity only three sessions taken at the beginning, middle and end of each training phase are shown. Black dots = successful vocalization trials; empty dots = vocalization trials in which SV occurred.

As expected, before training, the frequency of vocalization (filled circles) was low for both monkeys as indicated by the baseline mean percent of successful trials (Monkey 1, 4.4 ± 1.0%; Monkey 2, 14.1 ± 2.9%). The overall vocal performance increased with training, reaching a final value of 61.1 ± 6.8% in Monkey 1 and 64.4 ± 4.0% in Monkey 2 at the end of training.

Figure 8 shows the percentage comparison of coo-calls emitted during the ‘’Food’’ and ‘’No Food’’ intervals. Both monkeys had a significantly higher percentage of vocalization in the 30 seconds in witch food was presented on the board (“Food”) than in the subsequent trial interval (“No Food”).

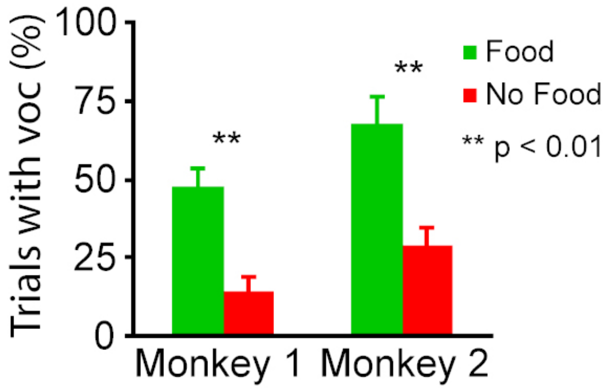

Figure 8 Percentage of trials with vocalization in “Food” and “No Food” conditions for the two monkeys. Error bars represent s.e.m. (standard error of the mean).

The statistical differences between the ‘‘Food’’ and ‘’No Food’’ condition periods indicate that monkeys have clearly distinguished the two phases of the task. Since the earliest signs of progression in vocal learning, we noticed that trained vocalization required considerable efforts in both animals. Indeed, they often failed in emitting any sound, while still producing the characteristic lips configuration that normally accompanies coo-call execution (Figure 9).

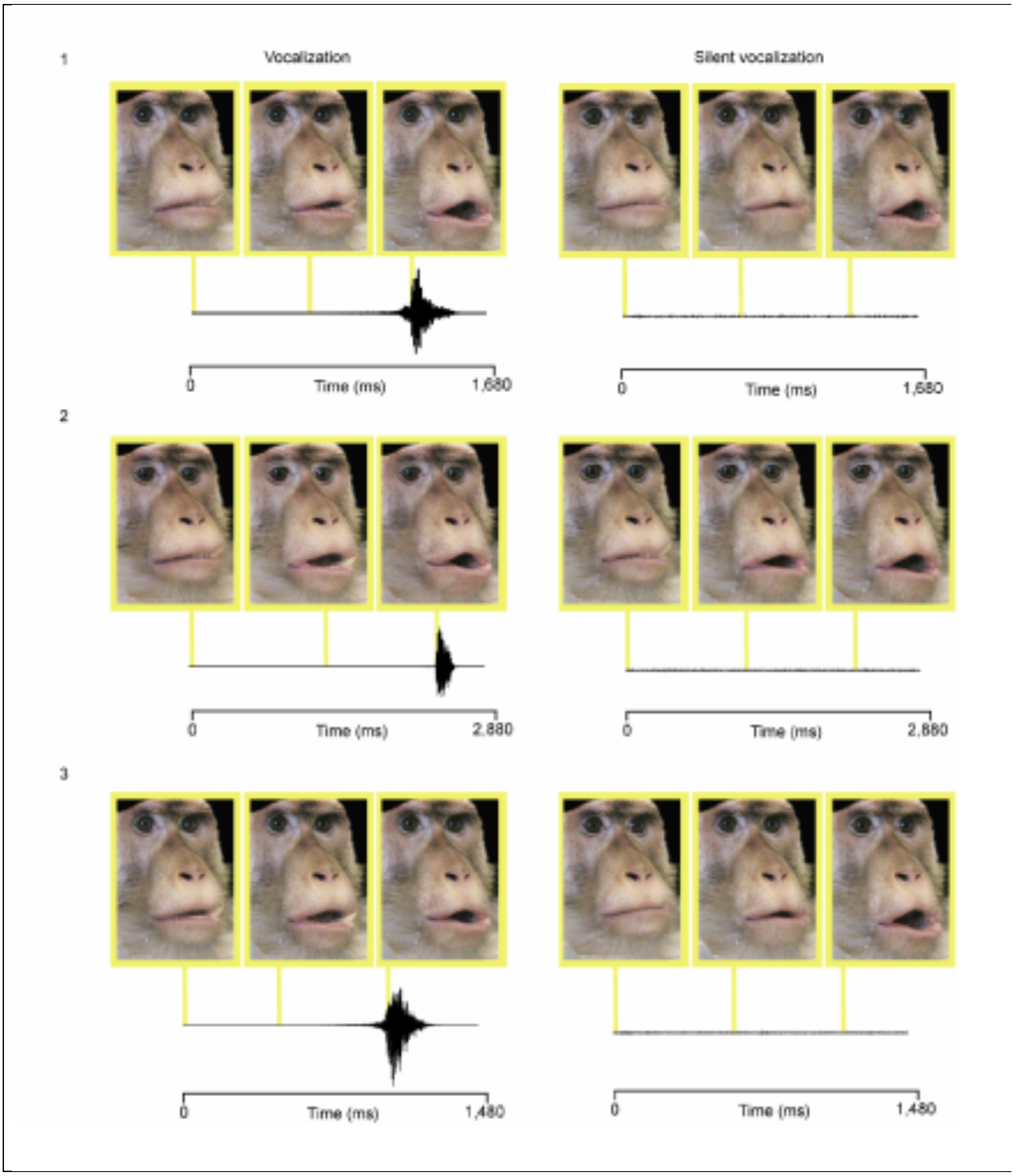

Figure 9. Lips configuration during coo calls and silent vocalizations. Three examples of sequences of coos and SVs are shown. Each example was taken during the same recording session. Note that coos and SVs involve similar mouth configuration and timing of unfolding. The third frame of each sequence corresponds to the maximal lips protrusion.

Interestingly, the percentage of SV (empty circles) made during the baseline phase was nearly zero. As training proceeded, the number of SV rose, together with the number of actual vocalizations, suggesting that this behaviour is a failed attempt to vocalize (see Figure 7). This idea is in line with the fact that this gesture does not correspond to any other known pig-tail macaque communicative facial expression and that it was never observed in untrained monkeys housed in our facility.

Neuronal responses during vocalization

Once the vocalization rate reached a criterion of 60%, a functional characterization of the PMv neuronal properties was performed in both monkeys. As described in the material and method paragraph the visual, somatosensory and motor properties were carefully investigated to locate the hand and mouth motor neurons representation (Figure 10, Left).

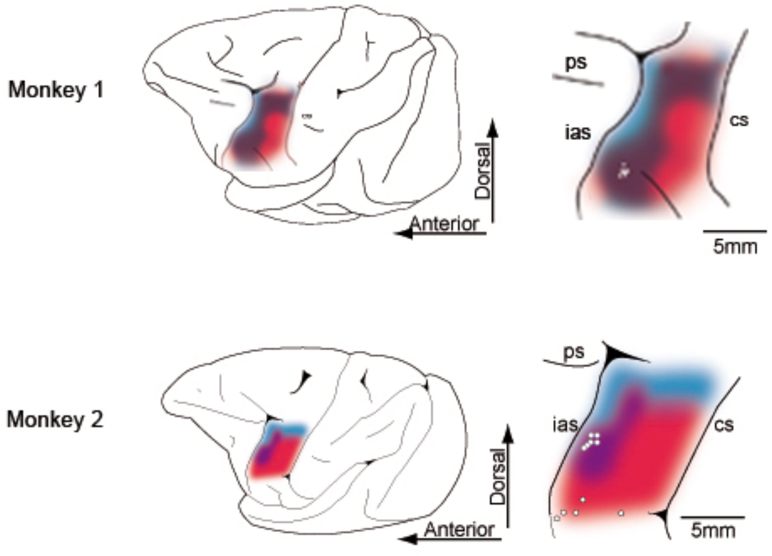

Figure 10. Recorded regions and vocalization-selective neurons. *Left.* Lateral view of the left hemisphere of Monkey 1 and Monkey 2. *Right.* Enlarged view of the recorded area showing the position of electrode penetrations where vocalization-related neurons were found (white dots). Note that some penetrations overlapped. Colored sectors indicate hand (blue), mouth (red) and overlapping hand and mouth (purple) motor representations. cs=central sulcus, ias=inferior arcuate sulcus, ps=principal sulcus.

During this investigation we identified sectors in which neurons correlated to vocalization could be found. A total of 106 neurons in 31 penetrations have been studied in order to assess whether the discharge was correlated with vocalization. Out of them, 63 neurons discharged in relation to vocalization. In fifty-one of them we recorded a sufficient number of trials allowing statistical analysis. All these neurons presented a significantly higher discharge during vocalization with respect to rest condition. Twenty-eight showed a selective discharge for vocalization (Table 1) when compared to other behaviours involving mouth movements.

	Before Sound Onset	During Sound Emission	All
Vocalization-selective	20	8	28
Vocalization/mouth related	16	7	23
Total	36	15	51

Table 1. Number of neurons responding during trained vocalization.

An example of the location of penetrations in which vocalization-selective neurons were found is given in Figure 10 (Right). In both monkeys their location appears to include the lateral part of area F5 (F5c) and, possibly, particularly in monkey 2, it extends in area DO. The area DO is located in the dorsolateral convexity cortex, at the level of the inferior precentral dimple (Belmalih et al 2009).

Figure 11 shows three examples of vocalization-selective neurons studied in different control conditions.

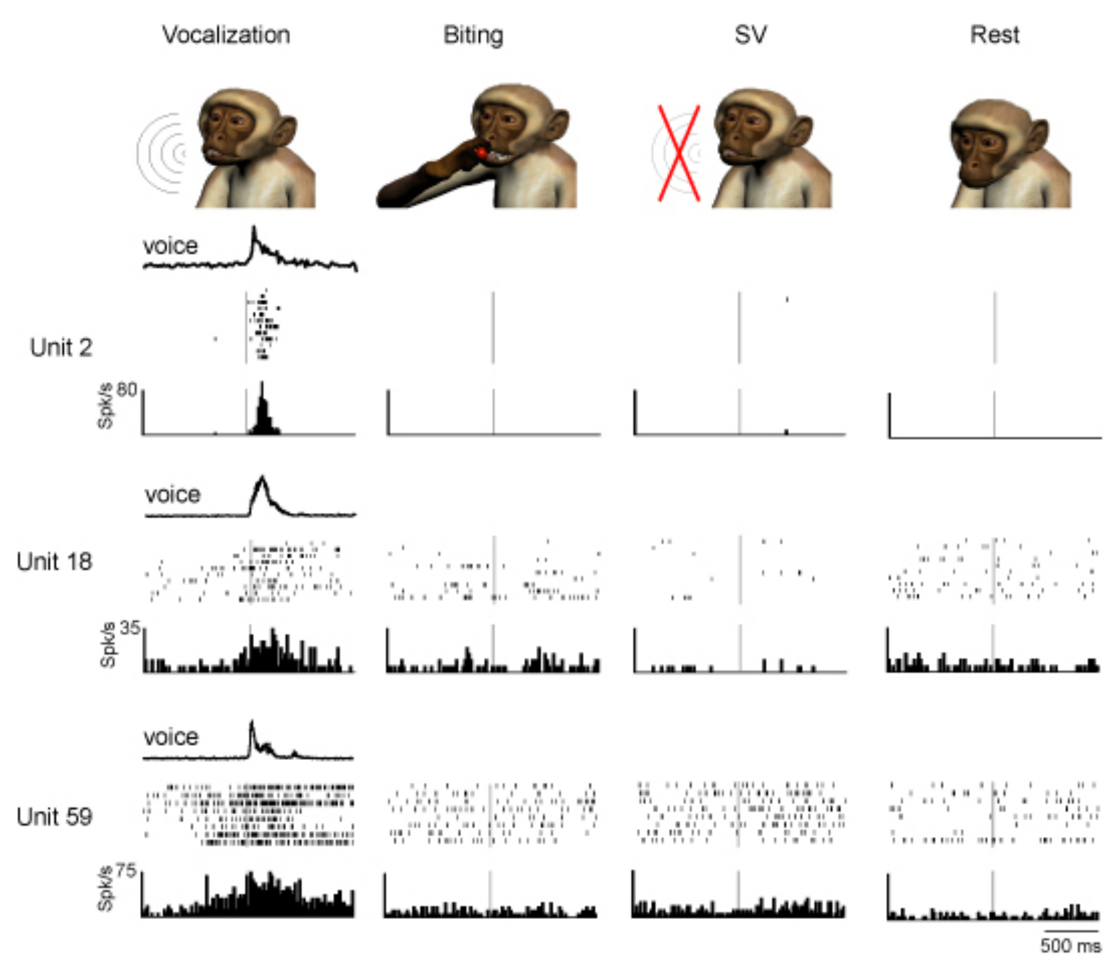

Figure 11. Examples of three vocalization-selective neurons recorded during four different behaviors. For each unit, rasters and histograms illustrate the neuronal discharge aligned (vertical gray line) with behavioral events. They correspond to monkey sound emission onset during vocalization, contact with food during biting and maximum lips protrusion during silent vocalization (SV). During rest, the activity alignment corresponded to the midpoint of a period in which the monkey did not produce any movement. The root mean square of sound trace of monkey vocalization is depicted for each unit above the vocalization raster

An analysis of the time course of the discharge of the vocalization-selective neurons showed differences in firing onset time with respect to sound emission. Unit 2 of Figure 8 is an example of neurons discharging during sound emissions, while Unit 18 and Unit 59 are examples of neurons whose activity started before the onset of sound emission. The distribution of firing onset of all vocalization-related neurons is shown in Figure 12.

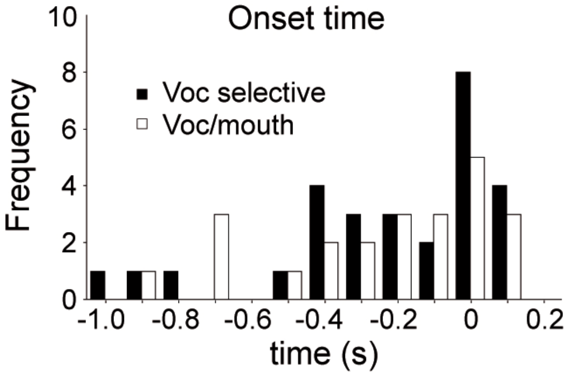

Figure 12. Classification and frequency of neurons according to discharge time onset with respect to the beginning of sound emission. Black bars: vocalization-selective neurons ; white bars: vocalization-related neurons .

The onset time of discharge may vary from 1000 ms to 50 ms before sound emission onset, but most neurons discharge in an interval of 400ms before the vocalization. Of the 28 vocalization-selective neurons 20 can be classified as anticipatory and 8 as having discharge during sound emission. In most of the recorded vocalization neurons the discharge started before sound onset (71% of vocalization-selective and 65% of vocalization-related).

Another interesting observation emerged from the study of the neurons of monkey 2. As already mentioned above and shown in Figure 10 (Right) vocalization-neurons were found in two groups of penetrations. The timing of the discharge of the two neuronal groups seems to reflect two different behaviours. Neurons located medially showed a discharge pattern well synchronized with vocal sound emission, while the lateral group displayed anticipatory discharges. A Pearson’s correlation coefficient test shows that the difference is statistically significant ($p < 0.05$) (Figure 13)

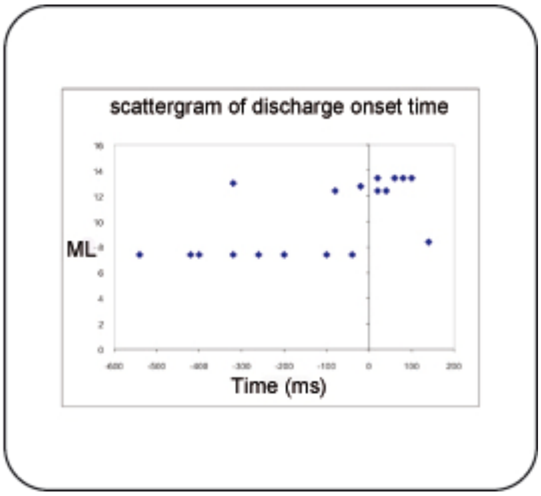

Figure 13. In the graph the blue dots represent the relation between timing of vocalization activity (X axis) and the medio-lateral position where vocalization related activity was recorded (Y axis). The time zero (vertical bar) corresponds to the onset time of the vocal-sound emission.

The discharge of vocalization-selective neurons does not depend on auditory feedback. In fact, we never recorded responses when the vocalizations were played back (see Material and Methods). Furthermore, tactile stimulation applied inside the mouth, on the tongue, face and anterior neck did not elicit any response, showing that also somatosensory feedback cannot justify the neural discharge.

Another interesting observation was carried out by comparing the neuronal discharge during during silent vocalization, and air blow emissions and during food calls production. Even if all this behavioural events apparently share similar oral movements, significant neuronal activity has been recorded only during the conditioned coo-calls emission. Some examples of that selective neuronal vocalization activity are shown in Figure 14.

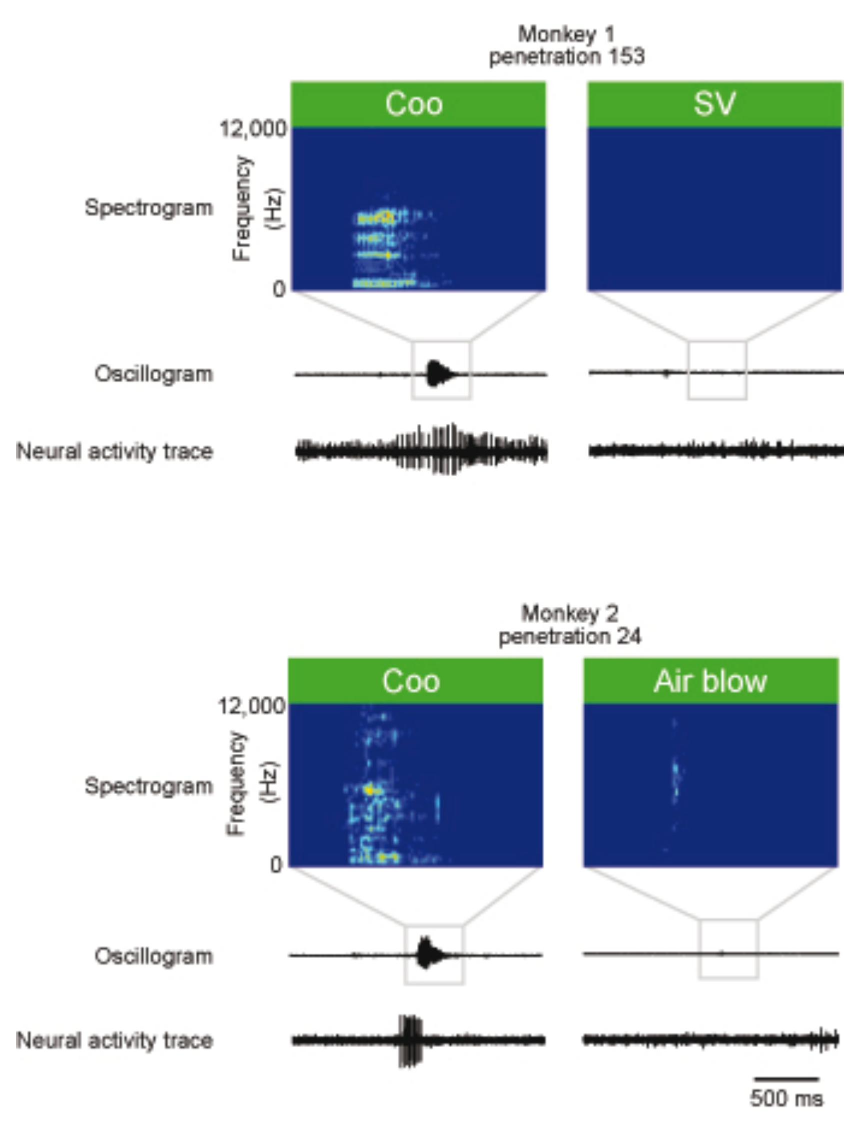

Figure 14. Neuronal activity and sound features related to different behavioral events during “Food” condition. **Top panel.** Coo and SV; **Bottom panel**. Coo and Air blow. Note that vocalization-related neurons do not fire during the emission of SV or Air blow.

We also verified whether vocalization-related neuronal activity was present during spontaneous vocalization (“No Food” condition). This is a difficult issue to address since spontaneous vocalizations were very few after the training period (Figure 15).

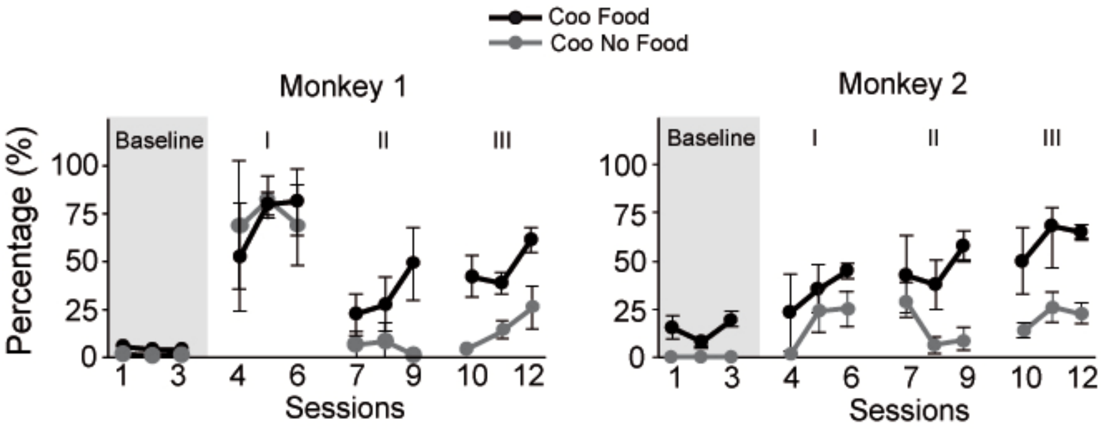

Figure 15. Monkeys vocalizations during Food and No Food throughout baseline and training phases. Each dot of the line graph represents the average percentage of the performance on three consecutive training sessions. For sake of clarity only the sessions taken from the onset, middle and end of each training phase are shown. Black dots = successful vocalization trials; grey dots = vocalization during No Food Interval.

Therefore, the comparison of single units recorded during both “Food” and “No food” vocalization would yield only few cases. To overcome this limitation, we performed a population analysis in which we compared the normalized root mean square (NRMS) of the raw neuronal activity trace during all coo-calls made by the two monkeys in the “Food” and “No food” conditions (n=320 and 37, respectively; see Materials and Methods for details). Figure 16 shows the time course of the mean NRMS neuronal activity in the two conditions.

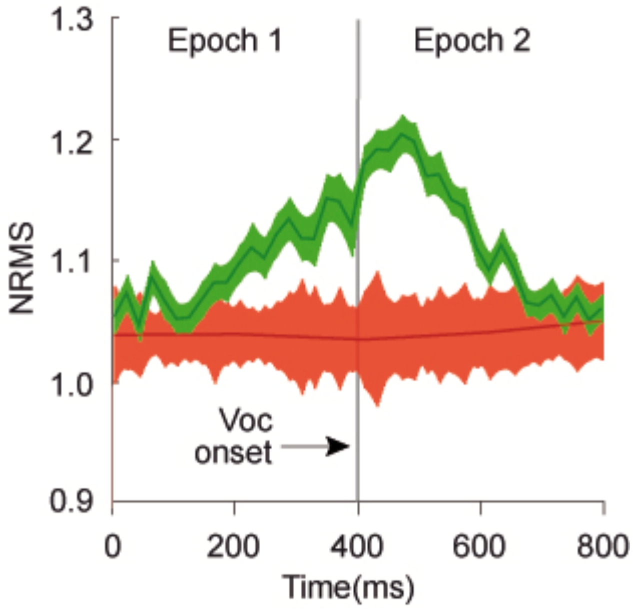

Figure 16. Time course of the population of NRMS of neuronal activity associated with “Food” (n=320) and “No food” (n=37) vocalizations. The dark green and dark red lines represent the mean NRMS of “Food” and “No food” conditions, respectively. The light shaded colors represent ±1 SEM.

Only “Food” condition presents an increased activity associated with call emission. A 2 X 2 ANOVA for repeated measure (Figure 17) showed a significant main effect of the factor Condition ($F_{1,72}$=18.92, P<0.0001), revealing that the neuronal activity associated with the coos made in the “Food” condition is significantly different from that recorded during the “No Food” one.

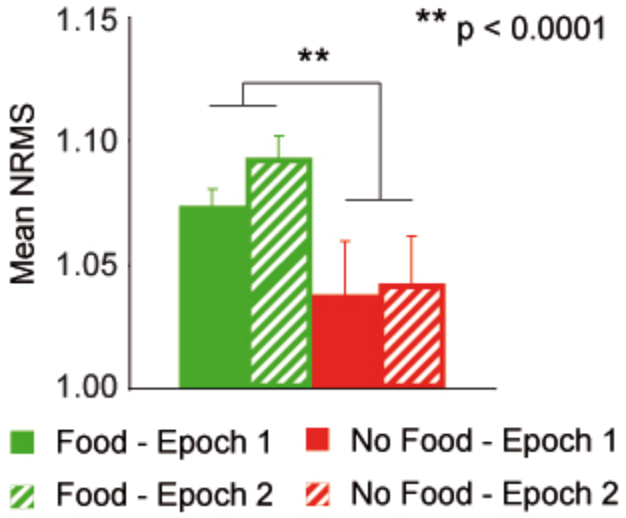

Figure 17. Histograms (means + SEM) showing the comparison between "Food" and "No food" NRMS calculated in the epochs before sound emission (Epoch 1) and during and after sound emission (Epoch 2)

In addition to population analysis, the neuronal specificity for "Food" vocalization was also directly assessed in nine neurons in which it was possible to directly compare the activity of the same unit in both "Food" and "No Food" conditions. Figure 18 shows examples of three different neural traces in the two conditions. It is clear that the discharge was present only for vocalizations emitted during the "Food" condition.

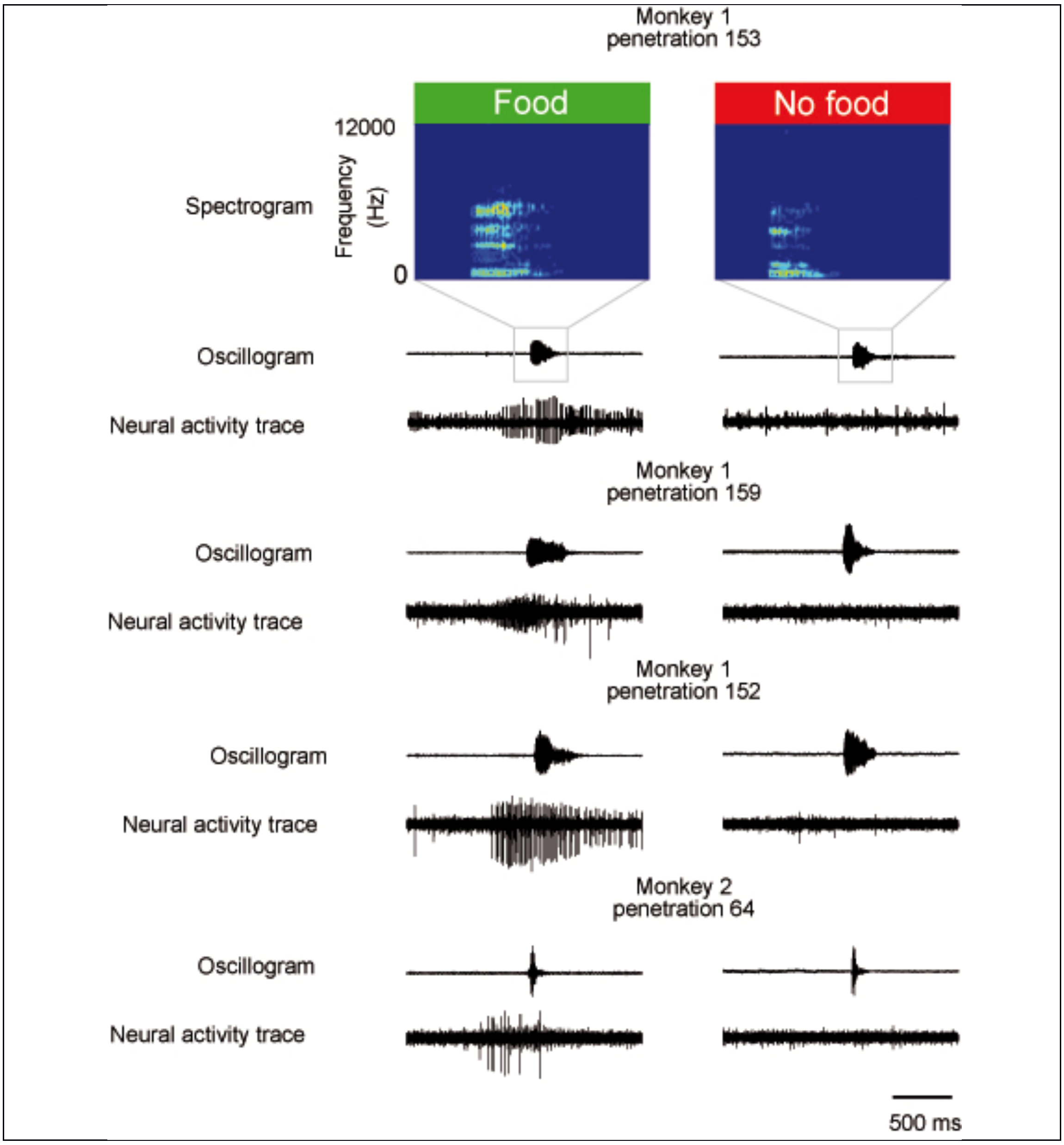

Figure 18. *Top panel*. Spectrogram examples of coo-calls emitted on “Food” and “No Food” conditions. *Bottom panel*. Neuronal activity and sound features recorded during food (left) and No-food (right) calls.

In order to verify whether this difference in discharge is related to a diversity in sound emission, we investigated sound characteristics in the two basic conditions of the task, by measuring the fundamental frequency (F0) and the first (F1) and second (F2) formants of the vocalizations. Figure 19 shows the mean F0, F1 and F2 measured in 30 coos for each condition in the two monkeys.

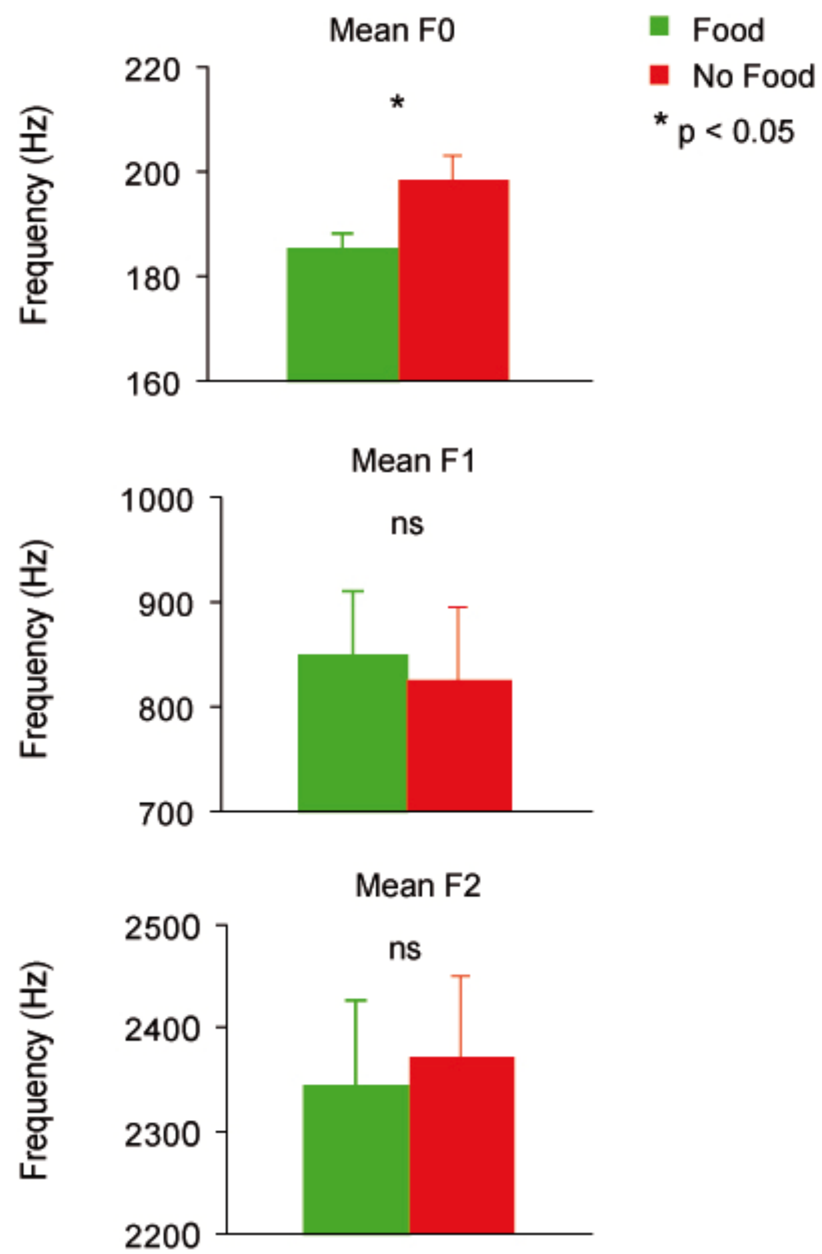

Figure 19. Comparison of mean F0, F1 and F2 of coos made during “Food” and “No food” conditions. Only F0 bears significant differences (paired t-test. P < 0.05)

The two conditions are significantly different in the F0 component (Z=-2.24, $p < 0.05$, two-tailed Wilcoxon paired-sample test) but not in F1 (Z=0.53, ns) and F2 (Z=-0.55, ns), indicating that the differences in sound emissions between conditioned and non-conditioned vocalizations imply different larynx movements but similar mouth articulation. Examples of the spectrograms of coo-calls recorded during individual trials in the “Food” and “No food” conditions are shown in Figure 20.

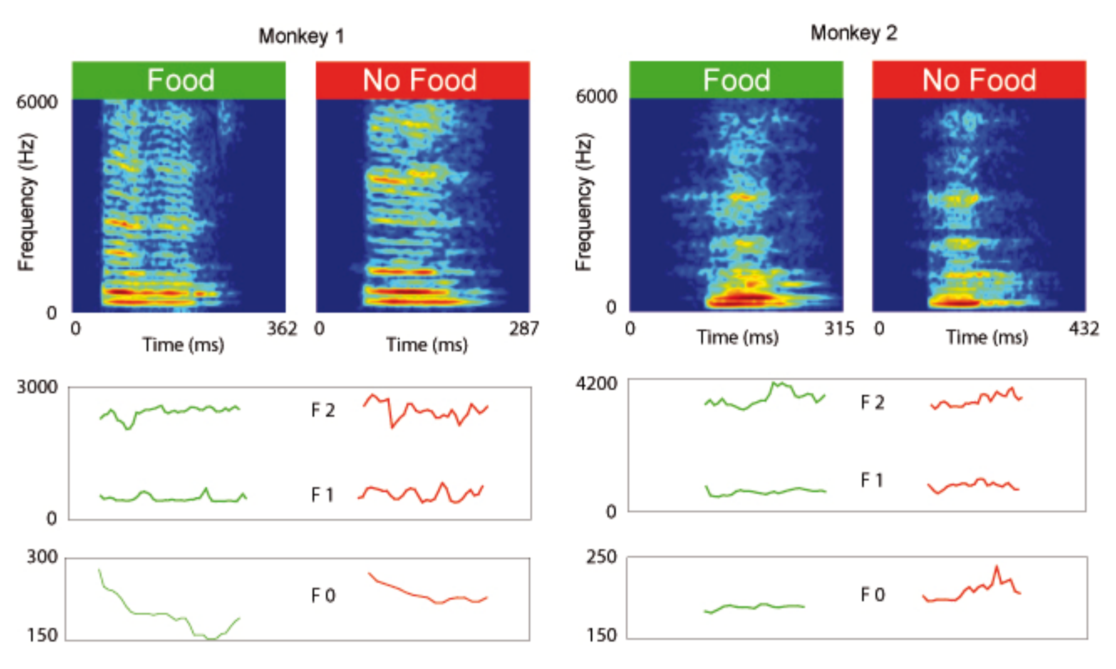

Figure 20. Examples of sonograms of coos made during “Food” and “No food” conditions. The differences in F0 and the similarities in F1 and F2 are illustrated in the boxes below the sonograms. In the x-axis the time scale is the same of the sonograms depicted above.

In order to directly verify whether vocalization-selective neurons could produce a motor output, we applied long-train electrical microstimulation to the sites where vocalization-related neurons were found (see Materials and Methods). Out of 71 stimulated sites (19 penetrations), 29 resulted to be excitable. In 22 of them, mouth movements (lips, jaw, tongue – mostly in combination) were elicited, while in 7 (4 penetrations) it was possible to elicit larynx cartilage movements together with tongue, jaw and lips movements (see Figure 21).

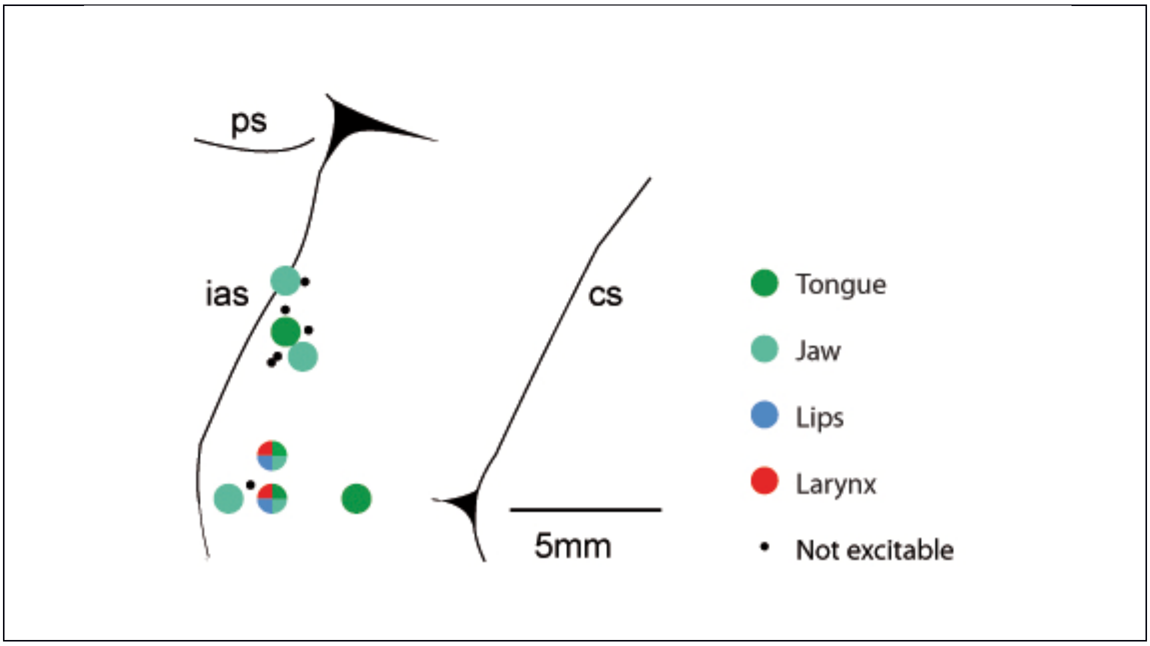

Figure 21. Representation of the penetrations in PMv in which long-train microstimulation evoked larynx, mouth and tongue movements. cs=central sulcus, ias=inferior arcuate sulcus, ps=principal sulcus.

The larynx cartilage movements elicited by the electrical microstimulation were observed both visually and tactilely.

Discussion

Behavioural results

Our results yield further confirmation that macaque monkeys can achieve a significant level of vocal control and learn to emit vocalizations voluntarily when submitted to a vocal operant conditioning task (Sutton et al., 1973; Aitken and Wilson, 1979; Hihara et al., 2003).

In the literature it is possible to identify cases where the conditioning of vocalization has failed (e.g. Aitken 1981, Aitken and Wilson, 1979, exp.1; Yamaguchi and Myers, 1972), but also numerous cases in which it was succesfull (eg Aitken, 1981, Aitken and Wilson, 1979, Hihara et al., 2003, exp.2; Chiao et al, 1994, Larson 1991, Larson and Kistler, 1984; Larson, Yajima, and Ko, 1994, Sutton et al., 1973; West and Larson, 1995; Yajima and Larson, 1993). Pierce (1985) has examined some of the factors that could affect the outcome of the training. He concluded that effects such as species, age and sex apparently are not such limiting factors on the control of vocal production, while the type of used reward is a limiting factor. However, according to Pierce, the main factor influencing the conditioned vocalization task results is the 'intraspecific' difference between individuals submitted to the training. An example to support that conclusion could be the Aitken's experiment (Aitken ,1981). Out of 12 rhesus monkeys, the authors have obtained good results with nine subjects, while three have failed, even if they were subjected to the very same procedure.

Available data show, however, that a vocal operant conditioning task has been successfully used to increase rates of vocal response in various animal species (e.g. cats, Molliver, 1963; rats, Lal, 1967; dogs, Salzinger and Waller, 1967; birds, Hake and Mabry, 1979). It is notoriously hard to achieve such results in nonhuman primates (see Pierce, 1985; Yamaguchi and Izumi, 2008). Indeed, previous studies reported that vocalization performance in macaque can remain low even after a long over-training period (Yamaguchi and Myers, 1972; Aitken and Wilson, 1979) and that it is very sensitive to context changes (Pierce, 1985; Yamaguchi and Izumi, 2008).

One of the most critical findings of the present work is the occurrence of SV, a behavior emerging during training in both monkeys. The dissociation between mouth and larynx displayed during the vocalization task is not surprising. While it has been shown that monkeys can be successfully conditioned to make oro-facial actions like tongue protrusion (Murray et al., 1991; Murray and Sessle, 1992), jaw movements (Murray and Sessle, 1992; Yoshino et al., 1998; Izumi et al., 2001), and lips protrusion (Louboungou and Anderson, 1987), literature about vocal training suggests a limited degree of vocal control (Pierce, 1985).

As discussed in the introduction, the limited degree of monkey vocal control has been attributed to the neuronal wiring of the vocal apparatus (Jürgens 2002, 2009). The Lack of direct neuronal connections between the motor cortical larynx neurons and the subcortical vocalization motor neurons interferes, according to the author, with a voluntary vocal production.

In synthesis, our results indicate that the larynx – at least with the scope of emitting vocalization – can be brought under partial voluntary control and, interestingly, the overall performance of our monkeys clearly reveals a dissociation between mouth and larynx control. This suggests that the cortical neural substrates, responsible for mouth and larynx control for the purpose of voluntary vocalization, as opposed to those involved in emotional calls, do not reach a full coordination in the macaque monkey.

Neurophysiological results

The main finding of the present study is that there are neurons active during conditioned vocalization in the lateral part of PMv. This same cortical sector also hosts neurons coding mouth motor acts such as licking, sucking and biting. This could raise the criticism that thevocalization related discharges are due to mouth movements. However, our data indicate that, in order to obtain a vocalization-related neuronal response in PMv, the complete vocalization action leading to sound emission is required, while complex mouth movements in the absence of vocalization are ineffective (vocalization selective neurons).

The mechanism underlying monkey vocalization action is similar to that of humans: the air exhaled from the lungs provides power to drive oscillations of the vocal folds (commonly known as vocal ‘cords’), which are located in the larynx. The rate of vocal fold oscillation determines the pitch, also known as fundamental formant (F0), of the produced sound. The generated acoustic wave passes through the vocal tract (the pharyngeal, oral and nasal cavities), that acts as a filter, and finally outward on the environment through the nostrils and lips.

According to our data, vocalization-selective neurons do not respond when the vocal tract configuration, particularly the characteristic lip configuration that normally accompanies coo-calls emission, is performed without any sound (silent vocalization, SV). In addition, the finding that no vocalization-selective neuron responds to air blowing (air expultion from the mouth) excludes their possible involvement in controlling expiratory acts *per se*.

The specificity for conditioned vocalization shown by some PMv neurons is further supported by their different discharge in the two conditions ("Food", "No Food"). As illustrated in the bottom part of figure 14 (see Results), there was no neuronal response when the monkeys emitted vocalizations during the "No Food" presentation interval. In the reported examples is, on the contrary, evident the increasing of neuron firing rate related to "Food" vocalization. One could argue that the vocalization-related discharge in the "Food" condition can be explained in terms of task outcome and/or reward expectation. Although some neurons in premotor cortex have been described as potentially modulated by a reward (Roesch and Olson, 2003, 2004; Buch et al., 2006; Pardo-Vazquez et al., 2008), this interpretation is not very likely, due to the wide spectrum of timing (anticipatory or time-locked) and pattern of discharge of vocalization-related neurons. Moreover, differently from previous studies, in the present work we did not record neuronal responses related to the reward cue presentation or to the achievement of the reward. The temporal pattern of vocalization-selective responses, which is always connected to vocalization, appears to be more compatible with the motor control of different phases of it. This is also in agreement with the general properties of the neurons of this area, which can code specific temporal aspects of motor acts (Raos et al., 2006).

We hypothesize that the neuronal discharge difference in the "Food" and "No Food" conditions relies on the *voluntary* nature of "Food" vocalizations that can be also revealed by their sound structure. Before sound emission, as mentioned above, the acoustic energy generated by vocal folds in the larynx is filtered. The filtering is accomplished by a series of band pass filters, which modify the original sound. Formants are determined by the length and shape of the vocal tract, and are modified by moving the articulators (tongue, lips, soft palate, etc.). It is important to stress that formants F1 and F2 are determined by the shape of the part of the vocal tract located higher than the vocal folds, that act as a filter, whereas the fundamental formant F0 is determined by the vibration rate of the vocal folds. (Fitch, 2000).

The results of spectrogram analysis, carried out to compare the sound structure of conditioned and spontaneous vocalizations, showed a difference in F0, but not in Formants 1 and 2, thus indicating that in the former there is a lower tension on the vocal folds in absence of differences in the articulators. This suggests that, in conditioned vocalization, PMv can easily control the articulatory component, but does not completely coordinate it with the vocal folds and expiratory components, as it would be predicted by the widely-accepted source-filter theory of speech production (Fant, 1960; Fitch and Hauser, 2004; Ghazanfar, 2008). According to this theory, the production of voiced signals involves independent contributions from the ‘source’ (larynx), and the ‘filter’ (vocal tract). The hypothesis of partial control of vocal folds and of their coordination with the vocal apparatus is also corroborated by the high frequency of SVs, in which only the articulatory component is present.

In a previous electrophysiological study carried out in the cingulate cortex of macaque monkeys (West and Larson, 1995), vocalization-related neurons have been found, intermingled with neurons coding oro-facial movements. The purpose of the study was to provide a more complete description of the location and discharge properties of anterior mesial neurons involved in facial/vocal behavior. The two tested monkeys were previously trained to increase their vocalization rate. The neurons recorded were classified in four groups: neurons related only to vocalization, neurons related only to jaw opening, neurons related to both vocalization and jaw opening and neurons related to other motor mouth activity.

Our findings show similar categories of neurons also in PMv. However, concerning vocalization-related neurons, there are two important differences between these two regions. First, most of the anticipatory units recorded in PMv kept discharging during sound emission, while those of the cingulate cortex stopped firing at sound onset. Second, while all PMv neurons are excitatory, more than half of cingulate neurons show inhibitory responses. These differences could be explained by different roles exerted by cingulate and ventral premotor cortex in vocalization. The first would be involved in action initiation, while the second would code the coordination of mouth articulatory acts and larynx movements aimed at sound production. According to this model (the "limbic vocal control pathway" proposed by Jürgens and presented in chapter 2 of this thesis), the cingulate cortex would play a “gating function” on the premotor cortical system (Jürgens, 2002). In fact, lesion studies showed that monkeys with part of the anterior cingulate cortex removed are incapable to initiate conditioned vocalization, despite their preserved capacity to emit spontaneous calls (Sutton et al., 1974, Aitken, 1981). The hypothesized role of PMv in coordinating mouth and larynx movements for the purpose of vocalization is evidenced in the present work, by the elicitation, as a consequence of microstimulation, of associated movements of the larynx cartilage and those of tongue and mouth. The cortical sector in which these movements were evoked could correspond in part to those in which other authors (Hast et al., 1974; Simonyan and Jürgens, 2003) described activation of larynx muscles in anaesthetized monkeys. In addition to the findings of these works, in the present study we provided critical information: the PMv excitable sites evoking larynx movements match the presence of vocalization-related neurons. Although microstimulation experiments indicated the possibility of a larynx motor control from PMv, neuroanatomical experiments did not identify direct connection of the lateral sector of PMv with nucleus ambiguous (Symonian and Jürgens 2003). This nucleus is known to be directly involved in the control of intrinsic and extrinsic laryngeal muscles movements. The intrinsic laryngeal muscles connect the different laryngeal cartilages with each other (Yoshida et al 1992) while extrinsic laryngeal muscles connect the larynx directly and indirectly with the surrounding structures (hyoid bone, sternum, and pharynx) and play a major role in phonation (Jürgens 2002). The results of combined stimulation and inactivation study suggest that the PMv control of phonation could occur indirectly through the reticular formation (Jürgens and Ehnreich 2007).

On the other hand, the lateral part of PMv has a direct control on the articulators (Morecraft et al., 2001). The direct connections between cortical PMv area and the peripheral motor facial nucleus have been observed after injections of anterograde neuronal traces in seven rhesus monkeys (Morecraft et al., 2001). In addition, low-threshold electrical stimulation of the lateral part of motor cortex elicits movements of the jaw, lips and tongue (McGuinness et al., 1980; Gentilucci et al., 1988; Huang et al., 1988). The involvement of this cortical region in direct control of facial muscles is not surprising because the whole lateral part of PMv is involved in different facial and mouth movements. It was, instead, surprising to find the presence of vocalization-related neurons in monkey PMv. Altogether, these findings suggest that PMv vocalization neurons could have a motor output on the subcortical structures that control vocalization, acting on both phonatory muscles and oro-facial articulators.

As previously described, some PMv neurons are active not only when monkeys execute actions but also when they listen to the sound these actions produce (audio-visual mirror neurons), (Kohler et al., 2002). Furthermore, a PET study carried out on rhesus monkeys indicates that PMv could be involved in auditory processing of specie-specific vocalization (Gil-da-Costa et al., 2006). However, vocalization-related neurons did not show specific responses when the recorded monkeys were listening to vocalizations of conspecifics and even to theirs own vocalization. The lack of acoustic properties suggests that other types of neurons located in a different subregion of PMv or outside it could be endowed with these properties and that motor and acoustic properties specific for vocalization are not yet fully coupled in the monkey PMv.

In accord with this hypothesis, recent neurophysiological and neuroimaging studies have revealed other cortical region involved in coding listened species-specific vocalizations (Ghazanfar et al 2005; Romansky et al 2002, 2005; Romansky and Averbeck 2009) among which the temporal lobe and the prefrontal cortex.

Rauschecker and colleagues found that 89% of lateral belt (part of the primary auditory cortex in temporal lobe) neurons responded to specie-specific rhesus monkey calls (Rauschecker et al., 1995). Furthermore, in a recent study Ghazanfar et al. recorded single-unit and LFP signals in the STS and in the lateral belt auditory cortex showing similar results (Ghazanfar et al., 2005, 2008). Other cortical regions within the anterior temporal lobe that receive projections from belt and parabelt auditory cortices are also involved in the processing of complex sounds including vocalizations (Poremba et al. 2004).

Other studies have shown that ventrolateral prefrontal (VLPF) auditory neurons do not readily respond to simple acoustic stimuli such as pure tones (Romanski & Goldman-Rakic 2002) but are robustly responsive to vocalizations and other complex sounds (Averbeck & Romanski 2004, Gifford et al. 2005, Romanski et al. 2005, Russ et al. 2008). VLPF auditory neurons are located in an area that has been shown to receive acoustic afferents from the anterior belt, the parabelt, and the dorsal bank of the STS (Hackett et al. 1999; Romanski et al. 1999a,b; Diehl et al. 2008). These sample studies have clearly shown that in the superior temporal and prefrontal cortices of the macaque there are neurons coding sophisticated, dynamic processing of information relevant to the behavioral/acoustic categories of species-specific calls. However, the possible motor properties of these neurons during vocalization were not investigated.

Altogether, these findings indicate that in monkeys the acoustic input reaching frontal areas might not be coupled with the motor representation of vocalizations, as it occurs in other species, such as humans and songbirds (Pulvermuller et al., 2006; Prather et al., 2008; Prather et al., 2009), in which several aspects of the species-specific vocalization are learned through listening and practice.

Conclusions

Several authors proposed, based on anatomical and functional evidence (see the introduction and BOX 1) that different parts of monkey PMv could be the homologue of human area 44 (Deacon, 1992; Rizzolatti and Arbib, 1998; Fogassi and Ferrari, 2007). The demostration of the presence, in the lateral part of area F5, of the vocalization-related neurons provides new data supporting this homology. This would imply that this region, beyond controlling the oro-facial movements involved in ingestive and communicative gestures (Ferrari et al., 2003), could combine this control with that of sound emission. The identification of neurons coding voluntary vocalization favours the idea that this type of coding could be a precursor of a volitional phonatory-based communicative system that dramatically expanded in the human lineage.

Several theories tried to explain at which point of human evolution communicative gestures were substituted by speech. Human vocal tract anatomy differs from that of other primates. Human larynx, even structurally unvaried, remains much lower in the human throat than in apes and monkeys. Anthropological studies (Crelin 1987; Liberman and Crelin 1971) argued that speech did not take place until the larynx descended during the *Homo sapiens* period. According to Lieberman and McCarthy (1999) the descending larynx increases the space available for articulation.

According to the gestural theory on language evolution, human language evolved gradually going back perhaps 2 million years to the origins of genus *Homo (*Corballis 2003*)*.

With the global shift to a cooler climate about 2.5 million years ago, much of southern and eastern Africa probably became more open and sparsely wooded (Foley, 1987). This left the hominids not only more exposed to attack from dangerous predators, but also forced to compete with them as carnivores. The solution was not to simply detect the predators presence and escape from them, but to establish what Tooby and DeVore (1987) called the ‘‘cognitive niche,” relying on social cooperation and intelligent planning for survival. Cooperation needed the ability to recognize dangers and to choose the best option to respond. *Homo* mental capacities were improved and well-defined and precise voluntary movements of hands and arms because at the beginning vocal communications were more at risk to be detected whereas manual gestures were silent. Manual ability started to be used to make tools. The involvement of hands in more activities allowed a major involvement of facial expressions in communication, so hands and face became an integrated system. Vocalizations gradually added to the gestural repertoire, achieving dominance, perhaps, with the emergence of *Homo sapiens* (Corballis 2003, 2010) who also presents an increase in brain size. When sound acquired a precise value and had to remain the same in identical conditions, this was most likely the cause of the emergence of human Broca's area from an area similar to monkey F5.

BOX 1

F5-Broca' s area homology

Broca’s region includes Brodmann’s cytoarchitectonic areas (BA) 44 and 45; they occupy the pars opercularis and pars triangularis of the inferior frontal gyrus (IFG) in the dominant hemisphere. They share the presence of very large pyramidal cells in deep layer III and in layer V, the lack of a clear border between layers II and III, and the low cell density in layer VI (Amunts et al 1999). However, whereas area 44 is “dysgranular” (containing a thin layer IV of small granular cells with pyramidal cells from deep layer III and upper layer V intermingled with those of layer IV), area 45 has densely packed granular cells in layer IV (“granular” area) (Amunts et al 2004; Matelli et al 1997). Cytoarchitectonic studies (i.e. studies of anatomical organization based on cell morphology and distribution) suggested homology between the disgranular area 44 in human and the area F5 in the monkey ventrolateral premotor cortex. (Petrides and Pandya 1994 and 1997).

The importance of the left-hemispheric area 44/45 in language production has been established since 1860s when Jean Paul Broca spoke about language disfunction associated to a lesion of this region (Broca's Aphasia). Research works carried out have established that Broca area is not simple motor-language production area, but it holds more articulated functions.

One of the first new capabilities explored in Broca's area was its involvement during execution and observation of hand/arm motor acts. The activation during execution and observation of intransitive motor acts is a property that defines mirror neurons (Gallese et al 1996) discovered in a monkey F5 ventrolateral premotor cortex. The presence of such neurons in Broca' s area was first confirmed by Grafton (1996) and could have been a key to suggest also functional homology between these two regions. Rizzolatti and colleagues (Rizzolatti et al 1996) observed that Broca’s region was activated also when the subject observed, imagined, and imitated the examiner using a precision grasp to enclose an object or to move his/her hand. Sequently Grèzes and colleagues (1998) corroborated that the observation of meaningful hand motor acts activated the left inferior frontal gyrus or Broca's region. One year later, another neuroimaging study investigated this motor aspect of Broca's area. Binkofsky et al. (1999) demonstrated a Broca's activation also during manipulation of complex objects. Jacoboni et al. (1999), in the same period, reported Broca's area activation in human brain during the execution and observation of fingers movements.

All these data attested, without any doubt, that in Broca's area, primary area for language production, there were neurons coding (in term of execution and observation) hand movements like monkey F5 .

But mirror neurons in monkey area F5 are also related to mouth motor acts and sound of motor acts as well, what about those properties in humans Broca's area?

Nishitani & Hari (2000, 2002) performed two studies in which they investigated imitation of grasping motor acts and facial movements, respectively. The event-related MEG was used. The first study confirmed the importance of the left IFG (Broca's area) in imitation. In the second study (Nishitani & Hari 2002), authors asked volunteers to observe still pictures of verbal and nonverbal (grimaces) lip forms, to imitate them immediately after having seen them, or to make similar lip forms spontaneously. During lip form observation, cortical activation progressed from the occipital cortex to the superior temporal region, the inferior parietal lobule, IFG (Broca's area), and finally to the primary motor cortex. The activation sequence during imitation of both verbal and nonverbal lip forms was the same as during observation. Instead, when the volunteers executed the lip forms spontaneously, only Broca's area and the motor cortex were activated.

During fMRI experiments, Buccino and colleagues (2001) investigated what happened during the subjects observation of videotaped-object and -non-objects related motor acts made through different effectors like hand/arm, mouth and foot. The results proved IFG activation also during mouth grasping motor act.

Buccino and colleagues few years later the grasp experiment above observed a Broca' s area activation when subject are watching others speaking even if no sound was emitted**. I**nterestingly, Broca's region was not activated when the human subjects watched a dog barking, i.e., a motor act that is not in the observer's motor repertoire (Buccino et al 2004). In a single pulse TMS (*Transcranial magnetic stimulation)* study, Fadiga and colleagues (1995, 2002) founded an increase of motor evoked potentials on tongue muscle when subject listening words containing syllables that require activation of the same muscle to be pronounced. These results revealed correlation between motor representations of heard words according to audio-visual mirror neurons in monkey F5.

Broca' s area and premotor cortex are active while listening sound associated with motor acts also in Watkins and Paus (2004) and Tettamanti et al (2005) experiment. Combining transcranial magnetic stimulation (TMS) and PET study; the authors observed that auditory speech activated the left IFG, suggesting that this area primes the motor system to respond to hear speech.

Furthermore anatomic asymmetry observation appears to support F5 and Broca's homology. The volume of histologically defined area 44 is larger in the left than in the right hemisphere, whereas area 45 is more sym-metric (Galaburdaet al 1980; Amunts et al 1999). Recent studies in great apes, have reported leftward asymmetries in the inferior frontal region corresponding to human Broca’s region (Cantalupo et al 2001, 2003 Hopkins et al., 2008), suggesting that the neuroanatomic substrates for left-hemisphere dominance in vocalization developed as early as five million years ago, long before speech emerged.

Finally, Hast and co-workers, using an electrical stimulation technique on monkeys, have shown that the lateral part of their premotor cortex, particularly F5, gets neurons controlling not only oro-facial activity but also larynx muscles (Hast 1974).

As reported, several studies support the homology between Broca's area and premotor area F5. In fact, both are involved during execution and observation of goal related hand and mouth motor acts. According to audio-visual mirror neurons in F5, Broca's area and premotor cortex are activated for listening to sounds associated with motor acts. Similarity with communicative mirror neurons, (neurons active when monkey observed facial expressions belonging to her own communicative expressions (Ferrari et al 2003) in F5, Broca’ s area activates when subjects observed another individual speaking without sound emission.

All this evidence could suggest that some aspects of language can be embodied in the sensorimotor system actually represented by mirror neurons.

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