Doctoral Thesis - XXVIII° Course
University of Parma
Department of Neuroscience

MIRROR NEURONS IN THE TREE OF LIFE
Development and Evolution of Sensorimotor Matching Responses

by

Antonella Tramacere

2015 - 2016

Supervisor
Pier F. Ferrari

External Supervisor
Atsushi Iriki

> He who loves practice without theory is like the sailor who boards ship without a rudder and compass and never knows where he may cast. —*Leonardo da Vinci*

General introduction

This thesis is a collection of articles written during my three years Ph.D. in Behavioural Biology - Neuroscience. These articles represent the development and evolution of an interpretation of mirror neuron system ontogeny and phylogeny in primates. Different articles try to inquire different perspectives of the same theme that is how discrete populations of mirror neurons develop during lifespan and what their evolutionary history might be.

Being defined as neural structures activated during both execution and perception of specific gestures (Di Pellegrino et al. 1992), mirror neurons recall a sensorimotor (or action – oriented) approach for studying brain and cognitive development, with a specific focus on how action and perception interwoven. An analysis of mirror neuron system ontogeny gives prominence to the range of perceptual stimuli and active exploration with the environment of the developing individual. However, other factors that do not seem *prima facia* linked to actions, such as the importance of genetic regulation and social stimuli that produce biases to cognitive processes, should not be dismissed. As such, an account of mirror neurons might potentially offer a comprehensive framework that contextualizes a range of important factors for brain and cognitive development of primates.

A description of mirror neurons, from their original discovery to the most recent studies, is reviewed in the introduction of each paper presented in this thesis. This will give the reader a general perspective of the theoretical issues that are debated within the field. Such debates are often polarized on accounts that emphasize dichotomic view on how mirror neurons are formed in the brain, and they often remain at speculative levels, without much analysis of the developmental factors and of the evolutionary mechanisms that in recent years have been unveiled, and that could help in interpreting possible evolutionary scenarios in mirror neurons formation.

Many accounts of brain development recognize the contributions from genetics, learning and social context (see Nešic & Nešic, 2014). The genetic – inherited components can be intended as quantitative biases, rather than whole dedicated cognitive processes. As such, genetics should not be considered as fixed and rigid programmes that drive the expression of behavioral patterns. In the context of primate mirror neuron system development, genes should be better seen as "start-up kits" that not only can lead, in some contexts, to flexible behaviour (through the contribution of regulation of genes transcription) (Müller and Wagner, 1996), but may also contribute to significant individual differences (Carey and Gelman, 2014; Frith, 2012).

Effectively, many adaptive mechanisms of brain development, honed by millions of years of evolution, are more strictly *genetically specified*. As for example, consider the neuronal circuits resulting from conservative developmental mechanisms orchestrating the segmentation of the vertebrates' hindbrain into compartment called rhombomeres. Specific transcription factors and Hox genes are differentially expressed in correspondent rhombomeres. Interestingly, *in vivo* analysis of neuronal groups after inactivation of one of those specific genes revealed distinct postnatal respiratory phenotypes, associated with various defects of central respiratory control. This and other findings (Borday et al. 2004) suggest that ground aspects of neuronal development and connectivity associated to very basic behavioural functions, such as respiratory rhythm, is provided by the activity of a number of key developmental genes and their control by regulative mechanisms (Chatonnet et al. 2002). In the same way, the emergence of first oral and pharyngeal motor sequences is under the control of the brainstem, that in turn is controlled by a family of conservative Hox and others transcription processes (Jacob and Guthrie 2000; Tsunekawa et al. 2005).

On the contrary, the ontogeny of other brain mechanisms seems to be less fix and more environmental influenced, although still somehow constrained. The development of various limbic and cortical regions of primates are still controlled by their anatomical location and genetic specification (Hager et al. 2012); however the characteristics, pathway of connections and firing pattern of distinct pool of neurons seem to be under the influence of *epigenetic regulation* (Striedter 2005; Fox et al. 2010). The development and function of these neural networks require, in fact, accurate gene transcription control in response to proper external signals (Krubitzer 2007). In this respect, epigenetic mechanisms, including DNA methylation, histone modification and other chromatin remodelling events, are more variable and critically instructive in mediating neural gene regulation[1].

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[1] It is worth to note that the distinction between *genetically regulated* and *epigenetic regulated* developmental brain processes does not imply a sharp separation between the activity of genes and their regulative control by peripheral sensorial systems. It does not mean that *genetically regulated* processes present not transcriptional control, neither that *epigenetic regulated* processes involve no specific genetic components. The difference lay instead on the *type* of molecular interaction. In the case of genetically regulated process, the epigenetic regulation occurs though highly stereotyped and conserved elements (Barber & Rastegar, 2010), while in the case of epigenetic regulated brain development the role of external input is more intrinsically instructive in producing specific cellular phenotypes (Fox et al. 2010). Another difference is that what we can call *genetically*

Similarly, certain important constraining factors to mirror neuron system development are more likely to be hard-wired and stereotyped. The emergence of facial movements and facial preference in primate newborns is a good example (Johnson and Morton 1991). From birth, newborns "track" the movement of a face-like stimulus longer than a control stimulus. This attentional bias makes them highly receptive to information from other individuals. Also, this is an example of how a strictly conserved behaviour (i.e. face preference in neonates) is action-oriented and could be instrumental for the emergence of more sophisticated cognitive processes such as those coupling execution – perception neural activity, described for mirror neurons. Face preference, in fact, involves not only "tracking" - moving the head to keep the stimulus, which itself is a moving target (Johnson and Morton 1991; Batki et al. 2000), but also "executing" – moving and orienting eyes and face (Batki et al. 2000). Furthermore, specific genetic factors underlying the level of neurotransmitters and variations in the expression of neurotransmitter genes are critically involved in these phenomena of social engagement. Serotonin, oxytocin and vasopressin allelic variants, in fact, positively correlate with variations of neural factors (e.g. amygdala activation and other limbic regions) that in turn are associated to variations in the engagement with the face and other social behaviour (Canli et al. 2007; von dem Hagen et al. 2011)

In the paper titled "Neonatal imitation and an epigenetic account of mirror neuron development" it is possible to appreciate a preliminary formulation of this perspective. This is the commentary to the paper of Cook et al. 2014 – published in *Brain and Behavioural Sciences Journal*. This commentary endorses that, the genetic and associative accounts previously proposed by some scholars for understanding the ontogeny of the mirror neuron system, has been extremely polarized on genetic *versus* plasticity or evolution *versus* learning. In sustaining that neonatal imitation and the nascent mirror neuron system are simply the result of associative learning, Cook and colleagues fail to consider the importance of neonatal differences in imitative abilities. Interestingly, variations in neonatal imitation in monkeys are correlated with visual attention to social partners (Heimann 1989), person recognition (Simpson et al. 2014) and face viewing patterns (Paukner et al. 2013), suggesting that variability in early social cognitive abilities may reflect genuine interindividual variations.

In trying to save the explanatory power of both accounts, the commentary focuses on the mirror neuron system associated to mouth gestures to propose a more integrative framework. Accordingly, mirror neuron system development has been interpreted as a combination of factors including the early specification of behaviour that is possibly evolutionary conserved

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regulated processes occur in highly constant environment and mostly before birth, while *epigenetic regulated* processes have a protracted development (Striedter 2005; Fox et al. 2010).

(not dismissing the importance of molecular factors but rather identifying their synergistic impact on action-perception processes), the active exploration of the environment and the associated socio – cultural interactions with other individuals. In other words, mirror system for facial perception is thought to involve an hard – wired visual preference for face - which facilitate a facial interactions with conspecifics (i.e. primarily caregivers), specific neural networks, which in turn overlap with those controlling face movements and whose tuning occur through experience, likely involving epigenetic mechanisms.

In line with this reasoning, the *TiCS (Trends in Cognitive Science)* opinion article "Mirror neurons through the lens of epigenetic" emphasizes the idea that the variability found in mirror responses during laboratories experiments in various macaque and human individuals, may be due to both interindividual endogenous variability and experiential differences in postnatal environment. The idea that mirror neurons possess a rather uniform pattern of discharge certainly recognizes the most apparent property of this matching mechanism. Nevertheless, the widely accepted notion of uniformity of mirror neuron properties does not take into account important properties of mirror neurons, which are evident in raw recording data, thus overlooking the heterogeneity of the factors implicated in mirror neurons development.

"Mirror neurons through the lens of epigenetic" also integrates a key concept to the interpretation of mirror neuron system ontogeny, i.e. *canalization*. More generally, canalization is a mechanism that narrows the range of developmental possibilities. It represents the bias that an organism has toward acquiring some forms of a trait, with a corresponding decrease in plasticity during ontogenesis (i.e., there are a greater number of developmental possibilities earlier, compared to later, in development). Canalization buffers traits against perturbation due to non-specific experiential influence and non-standard genetic variations (Dor and Jablonka 2001).

That the development of mirror neuron system in primate is interpreted as canalized or *experiential canalized* indicates that evolution promoted the course of development to be reliably influenced by specific stimuli, although variations are possible because of particular or unusual events. Accordingly, the mirror mechanism to mouth and hand actions in primate would be a case of developmental sensitivity *only* to environmental factors that are themselves invariant within the organism's typical environment of development (Ariew 1999). The emergence of mirror neurons circuits coding mouth and hand perception and execution can thus be interpreted as a developmental process that involve gene – environment interactions and specific timing of perceptual preference for mouth and hand – like stimuli, plus the active exploration of the developing individuals with her own and others' body.

The concepts of epigenetics and canalization complement each other. In the phenomenon of canalization, epigenetic factors (generated by developmental and social niche construction on genetic information) can contribute to the stabilization of cognitive/behavioral development over time (Gottlieb 1991), through molecular memory during cellular differentiation. This epigenetic regulation is at the interface between the genetic programming of premotor and parietal neurons and the differential developmental influences of species-specific environmental niches.

Furthermore, the concept of canalization recalls an evolutionary scenario, where the development of neurons responding both to observation and execution of mouth and hand actions are partly the result of evolutionary processes. The paper "Mirror neurons development and related evolutionary hypotheses" goes in this direction. Here, the hypotheses formulated in the first two manuscripts take a more evolutionary perspective. This book chapter, published within "New frontiers in mirror neurons research" by *Oxford University Press*, more explicitly proposes that mirror neuron system associated to mouth and hand movements are an integral part of our evolutionary history and have been maintained in phylogeny in virtue of their role in sensorimotor actions development and social cognition. The chapter hypothesizes potential mechanisms that possibly produced constraints and evolutionary changes in mirror neurons development underlying respectively neonatal imitation and tool – use.

Simultaneous activation of premotor, parietal, and sensory cortical areas for some basic biological movements are considered as experientially—canalized and facilitated during the very early phase of development. An evidence of this initial canalization comes from the presence of neonatal imitation for mouth actions both in monkeys and humans (Meltzoff and Moore 1977; Ferrari et al. 2006; Simpson et al. 2014). During early face-to-face interactions, the visual stimuli of facial movement perceived by newborns can activate family of genes, likely in subcortical structures, that are responsible for the organization of synapses of premotor and parietal circuits involved in the production of the same facial movement. The idea is that epigenetic regulation underlies these processes and correlate to progressively emerging cognitive functions in monkeys and in humans.

Another important evolutionary hypothesis of changes in the mirror neuron system over time regards the use of tools in primates. Tool – use in macaques recruits the same areas that are involved in hand actions, with the differences that the neurons responding to tool use are better tuned for this task after a prolonged period of visual exposure or sensorimotor training to tools (Ferrari et al. 2005; Rochat et al. 2010). Thus, tool-use mirror neurons can be the result of a co-optation and functional shift of pre-existing hand-grasping or hand-reaching mirror neurons, and our prediction is that epigenetic processes underlying these changes in the specific brain regions. Epigenetic regulation underlying the variability of mirror neurons system may produce variations on which evolution has operated during hominid phylogeny. Thus, mirror neurons plasticity, epigenetically based, may have been the field of new evolutionary outcome in old monkeys and humans (Iriki and Taoka, 2012).

The use of the concept of epigenetic, however, should not be taken in a strictly reductionist perspective. Instead, epigenetics only represents the mechanisms of gene – environment interactions underlying mirror neuron system development and also a source of their phenotypic stability. Further, it does not suggest that the development of the mirror neuron system can be understood in the absence of neurophysiological or sociocultural factors. Accordingly, the last two articles of this thesis consider the mirror neuron system in a more widen extent, focusing on the evolution of the ecological niche that contributed to stabilize mirror neurons through learning and interactions with others individuals. The importance of socio – cultural processes and niche construction has been emphasized in the paper "Faces in the mirror, from the neuroscience of mimicry to the emergence of mentalizing".

Published in a special issue devoted to the topic of "What make us human" in the *Journal of Anthropological Science*, this paper tries to highlight the relevance of facial processing in the evolution of human cognition. Connecting a change in social niche with selective pressure that have likely increased the 'robustness' of mirror neuron system development, means also emphasizing the role of behaviour and social experience in the process of mirror neurons phylogeny. Further, this is likely to complement the hypothesis of mirror neurons system at more basic (i.e. molecular and neural) levels. In fact, by postulating that natural selection may have operated by adjusting the parameters of neonatal behaviour and by facilitating associative learning between sensory and motor areas (resulting in a process of canalization), one can only provide a narrative history of how mirror neurons actually operate. On the contrary, an evolutionary account must principally attempt to answer the question of when and how a particular change happened in a species' phylogeny. Accordingly, "Faces in the mirror" tries to sketch specific hypotheses for the processes or mechanisms that may have favoured mirror neurons stabilization during evolutionary history.

Particular attention has been given to dynamics of face-to-face interactions in the early phases of development and to the differences in the anatomy of facial muscles among different classes of primates. The hypothesis is that increasing complexity in social environment and patterns of social development have promoted a specialization of facial musculature, of the behavioral repertoire related to production and recognition of facial emotional expression, and their neural correlates. With the increasing social demand during the transition to modern anthropoid primates about 40 million of years ago (Dunbar 2010), mirror mechanisms related to face expression may have become pervasive and extended to the entire life, from the very first phases of development to adulthood. Changes in social niches, such as the birth of multilevel society and more complex dynamics of parental and social bonding (Dunbar 2010) may have led to favor individuals more efficient in coordinating own facial and mouth movements in response to those of others (such as primarily caregivers). Further, it may have produced a stronger selective pressure on facial recognition and on the complexity of the neuroanatomical mechanisms controlling facial muscles. In others words, higher frequencies of face – to – face interactions may have tuned and coupled neural circuits for facial production and recognition, i.e. cortical and subcortical mirror mechanisms, and increased control of facial musculature related to eye and mouth movements.

The paper “Mirror neurons in the tree of life, mosaic evolution, plasticity and exaptation of sensorimotor matching responses” I present in this thesis, proposes an interpretation of mirror neuron system evolution as well. The elements of novelty that it introduces are several. First of all, it compares the properties of mirror neurons in primate and non-primate species and reviews the literature that has been neglected in previous manuscripts (i.e. mirror neurons in marmosets and in songbirds, as well as the neuroimaging and anatomical studies in chimpanzees).

Further, it systematizes the analysis of mirror neurons variability elaborated in the first articles. Although in fact mirror neurons seem to reflect a uniform and stable execution-observation matching system both within and across the species, a closer inspection suggests that a simple and preliminary distinction may help to clarify evolutionary history of mirror mechanisms, by taking into account their various dynamics of sensorimotor processing. Through the manuscript, mirror neurons have been thus classified using two unambiguous physiological criteria: the modalities of sensory input triggering the response, and the effectors involved in the motor output, obtaining different subtypes of mirror neurons, i.e. hand visuomotor, mouth visuomotor and audio-vocal.

The scenario that emerges is compatible with the hypothesis of a *mosaic evolution*. The mirror system is interpreted as a set of interrelated traits, each with an independent evolutionary history reflecting unique evolutionary processes. The dynamic of their phylogenetic emergence in the different species suggests that during evolution different types of action have been selected – producing various developmental dynamics of mirror mechanisms, slightly differential physiological properties and patterns of connections with other neuronal systems for each of them. Indeed, mirror neuron system seems to develop with different timing and dynamics in relation to the effector hand, mouth or vocal tract, and in relation to the species – specific environmental demands.

Accordingly, the paper tries to analyse the conditions (i.e. physiological and molecular mechanisms, as well as the ecological niches) that critically contributed to mirror neurons evolution. In particular, the evolutionary history of hand visuomotor mirror neurons appears to be the result of a series of events where a combination of uses, reuses, exaptations and further specializations might have occurred. The existence of neurons responding to the observation and execution of hand actions is associated with the evolution of the forelimbs, which in turn is related to the neural control of muscles and bones evolved for locomotion and only in a subsequent time for highly mobile forelimbs. In the manuscript is possibile to go through this evolutionary history up to the point that mirror neuron system become ready to play a role in the *guidance and perception* of visually coding hand movements according to learned rules, where grasping and tool actions are fundamental behavioural acquisitions, needed for the survival of individuals in their natural environments.

Another element of novelty of this study is the analysis of songbirds mirror neurons, which are populations of neurons activated by others' vocal sounds and likewise activated by vocal production both in humans and songbirds. This analysis tries to establish a relationship between vocal learning and audio – vocal mirror neurons. From an evolutionary point of view, this may provide support to the idea that songbirds and mammals could have co-opted a similar primitive neural structure with corresponding functional characteristics for the emergence of vocal learning (Bolhuis et al. 2010), which in turn give rise to mirror neurons as consequence of the associated process of auditory feedback. Thus, hypothesizing that the neural matching between conspecifics' auditory input and vocal output coincided with the emergence of the first form of vocal learning, the analysis of mirror neurons may provide some insight to the evolution of vocal communication through a focus on the mechanisms that are crucially involved in it.

From a methodological point of view, in developing such an empirical – based theoretical framework of mirror neurons ontogeny and phylogeny, I have utilized an analytical approach to the current literature that examines the ways in which the cognitive mechanisms supporting action and perception are connected. I investigated the emergence of the mirror neuron system in human and non-human species, by using the methods of Philosophy of Science and Theoretical Biology to integrate behavioural and neural data.

In particular, I have considered data related to mirror neurons, mirror neuron system and mirror mechanisms in the brain (definitions of these expressions has been offered through the manuscripts and in the *Glossary* of the paper *Faces in the mirror*) of different anthropoid and songbird species. Further, I have considered and compared properties and factors of sensorimotor development in those same and others (i.e. prosimians and non – singing birds) species. Finally, I have tried to interpret these heterogeneous and interdisciplinary data through a critical use of the instruments and concepts of Philosophy of Biology and the more updated conceptualizations of brain and cognitive evolution.

In the last decades, the central idea of genetic information has been complemented with that of developmental systems (Griffith and Stotz 2000). Theoretical studies of the changing concepts of the gene provided, in fact, a transition between the neo-Darwinian style of Philosophy of Biology (where the Central Dogma of Molecular Biology by James Watson and Francis Crick was a key construct) and the kind of understanding of what is now called *evo – devo* (evolutionary developmental biology) (Amundson 2008). In particular, *evo – devo* have coincided with an investigation of the properties and implications of developmental dynamics, with an attribution of more explanatory power to developmental concepts (i.e. phenotypic plasticity, environmental scaffolding, constraints, exaptation, etc.) and a careful consideration of how these concepts integrate and complement with inheritance, variation and natural selection (Grisemeier 2014).

In the neo – Darwinian tradition, the emergence of specie – specific cognitive traits was considered as the result of natural selection (thus as an adaptation) or as the result of the cultural environment (Lorenz 1965; Williams 1966; Wimsatt 1986). *Evo – devo* adds to this tradition that multiple and reciprocal interactive mechanisms may play a role in producing regularities in brain – behavioural traits, such as phylogenetic and developmental constraints, environmental canalization, and reciprocal interactions between the various elements of the developmental system (Gould & Lewontin 1979; Wimsatt 1986; Gottlieb 1991; Griffith & Stotz 2000).

The emergence and origin of mirror neurons and associated sensorimotor behaviour has been analysed and interpreted within this integrative perspective. Specifically, particular attention has been given to the developmental dynamics of mirror neurons formation and to the jointly effects of their different developmental (physiological and socio – cultural) precursors, trying to avoid the classical dichotomy between innate - acquired or genetic – environmental. As consequence, a more complex perspective has emerged. This perspective is strongly convergent with recent work in psychology and cognitive neuroscience (Hepper et al. 1993; Kramer et al. 1996; Leppänen and Nelson 2009), which show the crucial role of external 'scaffolding' and perceptual bias in brain - behavioural development. This means that neural and cognitive processes (including those which can be explained in evolutionary terms) are constructed in each generation through the interactions of a range of developmental resources and through the constraining action of physical and cultural elements.

Evo – devo conceptualizations have provided a robust theoretical framework for the analysis of mirror neurons evolutionary history, as well. If *evo – devo* arose as a result of the impetus provided by the publication in 1977 of "Ontogeny and Phylogeny" by Stephen J. Gould - who reminded us of the importance of *heterochrony* (change in timing of development) as a mechanism for evolutionary change (Hall 2003), then mirror neurons phylogeny fully belong to the list of phenomena that can be interpreted within the *evo – devo* lens.

Specifically, monkey, ape and human beings seem to be endowed with mirror mechanisms since the neonatal phase as crucial correlated of affiliative and social interactions face – based, whereas prosimian primates seem to completely lack these types of behavioural phenomena. Thus, the comparative analysis of neural mechanisms (where available) and associated sensorimotor behaviour in anthropoid and prosimian primates may highlight important differences related to timing of sensorimotor development, and more specifically to how cortical and subcortical networks evolved in order to sustain mirroring behaviour in specific windows of development (for a detailed analysis of this issue, please refer to the paper "Faces in the mirror" reported in this thesis).

Finally, recent revisions of evolutionary theory justify the frequent resort to epigenetics in order to explain mirror neurons development and to unveil features of their evolution. In an *evo – devo* paradigm, in fact, epigenetics is thought to be the instrument to explore mechanisms of the emergence of specific traits during ontogeny and to compare their change during evolution (Müller 2008).

Broadly defined, epigenetics is the study of developmental interactions or mechanisms that are responsible for the emergence of a particular phenotype (Løvtrup 1981; Holliday 1990). Strictly defined, epigenetics refer to pattern of methylation and other chromatin markers of the genes (Bird 2002). Accordingly, the broad epigenetic perspective on mirror neurons suggests that it is important to understand key molecular and environmental factors, plus their differential timing of interactions in mirror neurons development. The strict perspective predicts that the physiological role of mirror neurons can be reflected at average at the epigenetic – nuclear level in specific brain regions, in line with evidences and models stating that epigenetic regulation associated to developmental plasticity reflect adaptive functional interactions of the brain with the environment during ontogeny (Fishell and Heintz 2013; Bronfman et al. 2014; Lokk et al. 2014).

It is worth of noting that the broad epigenetic perspective on mirror neurons may sound somehow equivalent to various learning accounts (Keysers and Perrett 2004; Cook et al. 2014) that have been proposed for explaining mirror neurons formation. It is not. The epigenetic perspective in fact moves beyond this learning approach by including not only top – down (socio – cultural) influences on mirror development, but also likely bottom – up (sub – cortical) effects (Bonini 2016) on their tuning and presence from early development. In addition, although fully considering environmental and socio – cultural influences on their origin and development, the epigenetic view doesn't dismiss mirror neurons role in the evolution of non - human and human primate cognition.

In sum, this doctoral work contextualizes properties and factors of different types of sensorimotor development in an evolutionary perspective, examining how mechanisms of learning evolved and are organized to produce adaptive specializations. This seems to be a necessary step in neuroscience and cognitive science, given that progresses in evolutionary theory are continuing to emphasize the crucial role of brain plasticity and its constraints in the evolution of cognition.

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Commentary

Brain and behavioural sciences

Neonatal imitation and an epigenetic account of mirror neuron development

Prepared in 2013 – Published in 2014

Elisabeth A. Simpson, Nathan A. Fox, Antonella Tramacere, Pier F. Ferrari

A number of lines of evidence support the notion that neonatal imitation is a real phenomenon.

Though we realize that it is unlikely our commentary will settle this debate, we believe that Cook et al. fail to consider the importance of individual differences in neonatal imitation. Neonatal imitation has been demonstrated using more than one gesture (which is critical because it shows specificity in matching) in over two-dozen studies. In fact, recent work—not reported by Cook et al.—refutes the notion that neonatal imitation is simply an arousal effect (Nagy et al. 2012). Similarly, neonatal imitation is not a reflex-like behavior, as newborns appear to remember, after a delay, both the particular gesture (Paukner et al. 2011) and person (Simpson et al. under review) with whom they interacted, and initiate interactions. Moreover, nursery infant monkeys, who have no exposure to contingent behaviors from caregivers, and therefore have no opportunities to learn to imitate, still show neonatal imitation (Ferrari et al. 2006). Given that neonatal imitation occurs in a variety of primates, it may be a shared behavioral adaptation (Paukner et al. 2013).

Critically, neonatal imitation may reflect activity of the nascent mirror neuron system, as it is associated with suppression of specific electroencephalogram (EEG) frequency band activity (Ferrari et al. 2012). This work is consistent with a recent study based on simultaneous EEG and functional magnetic resonance imaging in human adults showing activity of the parietal and premotor/motor cortex (i.e., mirror neuron areas) linked to EEG suppression within the alpha band (i.e., mu rhythm) (Arnstein et al. 2011). And, there is EEG evidence of a functioning mirror neuron system from birth in neonate macaques who lack any early face-to-face contingent experience with social partners (Ferrari et al. 2012).

Inconsistent neonatal imitation findings may be the result of variation amongst infants in imitation, indicating significant individual differences in infants' abilities to learn contingent behaviour, upon which critical cognitive and social skills are based (Reeb-Sutherland et al. 2012). In support of this idea, recent findings reveal individual differences in neonatal imitation in monkeys are correlated with visual attention to social partners (Simpson et al. under review; similar findings in humans: Heimann 1989), person recognition (Simpson et al.

under review), face viewing patterns (Paukner et al. 2013; Paukner et al. under review), deferred imitation (Paukner et al. 2011), and goal-directed movement (Ferrari et al. 2009). Therefore, rather than dismissing neonatal imitation —as Cook et al. appear to do—we argue that one should focus on the causes and consequences of individual differences in neonatal imitation through longitudinal (Suddendorf et al. 2012) and comparative (de Waal and Ferrari 2010) studies of newborns. We suggest that it would be insightful to examine neonatal imitation in infants who have siblings with autism spectrum disorder, a high-risk population (e.g., Chawarska et al. 2013), or examine effects of early experiences on neonatal imitation, including behavioral (e.g., Sanefuji and Ohgami 2013) and pharmacological (e.g. Tachibana et al. 2013) interventions.

In addition to their questionable view of neonatal imitation, we believe that Cook et al. are mistaken in opposing genetic and learning views on mirror neuron system development. Instead, like any developmental phenomenon, it is important to consider gene expression in different environments, and in different species, in order to understand how evolution produced predictable, functional, and species-specific phenotypes. Using this approach, we can examine how mechanisms of learning evolved to produce adaptive specializations through epigenetic mechanisms (Domjan and Galef 1983). Epigenetics is the study of changes in gene expression as a consequence of an organism's response to different environmental stimuli; genes can be temporally and spatially regulated and epigenetics is the study of these reactions and the environmental factors—including the prenatal environment—that influence them. Countless examples emerging from the field of epigenetics demonstrate that genetic and epigenetic inheritance is not indicative of innateness, nor are phylogenetically inherited traits insensitive to experience (e.g., Jensen 2013; Roth 2012). Indeed, epigenetic models now focus on the origins of complex behaviors; we propose that such models should be considered along with associate learning mechanisms in predicting developmental trajectories, within and between species. We agree that it is misleading to think that natural selection selects only specific 'good' genes.

Instead, natural selection acts on phenotypes, which are the result of complex interactions, including environmental effects on gene expression. Therefore, it is more fruitful to identify epigenetic regulatory factors responsible for the emergence of predictable developmental brain/behavior trajectories, than to search for genes that produce specific phenotypes. For example, in macaque infants, we are now beginning to understand the epigenetic mechanisms that can explain how early social adversity increases the risk of disease and disorder (e.g., Provençal et al. 2012). We also agree with Cook et al. that learning likely shapes the development of the mirror neuron network in the brain, but learning occurs differently as a function of individual characteristics and context. Selection pressures operate not only on the final phenotype, but also on the interactions between genes and the environment and the interactions between molecular factors and the environment (Bleckman et al. 2008). It is possible that mirror neurons evolved to support learning of basic functions of sensorimotor recognition of others' behavior, essential, though not specifically an adaptation for, higher order cognitive functions as well as sensorimotor learning (Bonini and Ferrari 2011). The interaction of genes and experience through learning can only occur if the basic neural circuitry is there to support such learning. We contend that mirror neurons may provide the scaffolding for these interactions early in life, having themselves been remodeled by epigenetic processes across evolution (Tramacere et al. in preparation).

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Tramacere, A., Ferrari, P. F., & Iriki, A. (in preparation). Mirror neurons, an epigenetic perspective.

Peer – review paper
Trends in cognitive sciences

Mirror neurons through the lens of epigenetics

Prepared in 2013 – Published in 2013

Pier F. Ferrari, Antonella Tramacere, Elisabeth A. Simpson, Atsushi Iriki

Abstract

The consensus view in mirror neuron research is that mirror neurons comprise a uniform stable execution – observation matching system. In this opinion article, we argue that, in light of recent evidence, this is at best an incomplete and oversimplified view of mirror neurons, whose activity is actually quite variable and more plastic than previously theorized. We propose an epigenetic account for understanding developmental changes in sensorimotor systems, including variations in mirror neuron activity. Although extant associative and genetic accounts fail to consider the complexity of genetic and non-genetic interactions, we propose a new Evo-Devo perspective, which predicts that environmental differences early in development, or through sensorimotor training, should produce variations in mirror neuron response patterns, tuning them to the social environment.

Introduction

Variation and plasticity in mirror neuron response properties (*see Glossary*) have been observed in neurophysiological experiments in awake primate brains, establishing equivalence between actions of the self (by execution) and actions of others (by observation). These neurons were first discovered in the ventral premotor cortex (area F5) and subsequently in the anatomically connected area: PFG of the posterior parietal cortex (Di Pellegrino et al. 1996; Gallese et al. 1996; Fogassi et al. 2005). The most striking property of mirror neurons is that they fire while monkeys are executing a goal-directed movement (i.e., grasping) and when observing the same, or similar, actions performed by other individuals. Therefore, mirror neurons are capable of mapping the visual description of biological meaningful events into the corre-sponding cortical motor representations. The straightfor-ward ‘execution–observation matching’ phenomenology interpretations of mirror neuron function have been useful in a wide range of disciplines in proposing uniform neural mechanisms primarily in the social domain of psychologi-cal phenomena – e.g., action understanding and imitation (Rizzolatti and Craghiero 2004)– but also spoken and sign languages, mind reading (Gallese 2007), and social disorders, including autism (Iacoboni and Dapretto 2006). Most researchers, while discussing the nature and function of mirror neurons, report what is considered the main characteristic of mirror neurons: namely, their matching mechanism. The idea that mirror neurons possess a rather restricted and uniform pattern of discharge is a widespread opinion that certainly recognizes the most apparent property of the matching mechanism.

Nevertheless, it overlooks the variety of responses that were originally described and discussed in the first papers describing mirror neurons, and that are informative for understanding the nature of mirror neurons. Recent work has also shown that the visual discharge of mirror neurons can vary depending on several contextual features, such as the observed end-goals of the agent, the space where the action is performed, the attention of the monkey, and the type of object grasped by the experimenter (Fogassi et al. 2005; Caggiano et al. 2011; Caggiano et al. 2012; Ferrari et al. 2005; Yamazaki et al. 2010). This work has also demonstrated that prolonged visuomotor experience affects the tuning of mirror neurons to others' actions performed with a tool (Rochat et al. 2010). The uniformity of the properties of mirror neurons has been claimed to develop through either associative (i.e., ontogenetic adaptive learning processes) (Heyes 2010) and/or genetic mechanisms (i.e., phylogenetic natural selection processes) (Cook et al. 2014; Del Giudice 2009; Bonini and Ferrari 2010). Further, in the genetic account, canalization mechanisms (Del Giudice 2009) have been proposed to contribute to the streamlining of associative learning to form sensori-motor matching for particular sets of preprogrammed body-part actions. In this opinion article, we argue that the widely accepted notion of uniformity of mirror neuron properties does not take into account important properties of mirror neurons, which are evident in raw recording data, thus overlooking the subtle, yet crucial, variations of mirror neurons. This is a matter for concern because it may lead to an over generalization of the roles of mirror neurons among psychologists, and even neuroscientists, who mistakenly require too much response stability, leading many to ignore such variations as mere outliers or noisy fluctuations. Thus, although there are indeed basic response properties of mirror neurons (i.e., visuomotor matching), at the same time they may possess critical variations and plasticity, which could be explained if mirror neuron response properties are formed through plastic biological processes during postnatal development. Here, we propose recently emerging epigenetic mechanisms as strong can-didates for subserving such processes, incorporating associative and genetic accounts (including canalization). Epigenetic mechanisms refer to DNA's differential expres-sion of proteins as a consequence of environmental influ-ences (at cellular, tissue, and whole organism levels). Gene expression can be switched on and off by several epigenetic mechanisms (*Box 1; Figure 1*) that, at the brain level, can ultimately affect how neurons connect to each other to produce and stabilize functional brain architecture. During the past few years, evidence has accumulated showing that environmental conditions influence epigenetic codes more than they influence the DNA sequence, making these codes suitable for supporting organisms to adapt to changes in the social and physical environments, especially during development. Small differences in epigenetic patterns can produce significant impacts on the phenotype, as demon-strated by studies on cloned animals and monozygotic twins (Fraga et al. 2005; Rideout 2001).

In what follows, we first discuss some immediate problems that appear to derive from an over-simplified vision of mirror neurons' properties, and then propose an *epigenetic account* which, by giving emphasis to the adaptive developmental stages of plasticity (or Evo-Devo mechanisms), establishes mirror neurons as biologically plausible phenomena, incorporates critical aspects of associative and genetic accounts, and is consistent with the remarkable variations of mirror neurons' properties. In the final section, we provide examples that represent subtle yet crucial variations of mirror neurons' properties, that tend to be overlooked by general readers, but that are well recognized by experimentalists who directly observe raw recordings of mirror neurons.

In this opinion paper we try to provide a coherent picture of how a rather simple sensory-motor mechanism might emerge in development, and how an epigenetic view might stimulate more ‘brain-based’ realistic experiments to predict specific neurodevelopmental outcomes and evolutionary-based explanations of mirror neurons' origins and functions.

Problems with current interpretations of the development of mirror neurons

The *associative account* posits that mirror neurons are a product of associative learning (Heyes 2010; Cook et al. 2014; Cooper et al. 2012). Through Hebbian learning, visuomotor neurons' response to the observation of others' hand actions might emerge in the parietal and premotor cortices during an early phase of development in which infants' sight of self-reaching towards an object is systematically associated with the motor command for grasping. The simultaneous firing of these neurons can strengthen visuomotor connections. Through this mechanism, perceptual and motor experience related to own-action could produce premotor and parietal neurons that simultaneously receive specific visual input from the STS region of the temporal cortex and potentiate the motor pattern that is related to grasping execution in parietal/premotor areas. Though persuasive, this model of mirror neuron development has important limitations. One such limitation is the “correspondence problem,” which refers to the problem of how newborns link visual input of others' facial gestures to their own motor representations of the same gestures, since infants cannot see their own face. This link appears to be present prior to any experience, as evidenced by human and macaque neonatal imitation (Meltzoff and Moore, 1977; Ferrari et al. 2006), making it difficult to explain neonatal imitation from a purely associative learning perspective. In macaques, infants imitate even in the absence of any prior experience of contingent facial interactions with caregivers, as infants in these studies are reared in a nursery from birth (Ferrari et al. 2006).

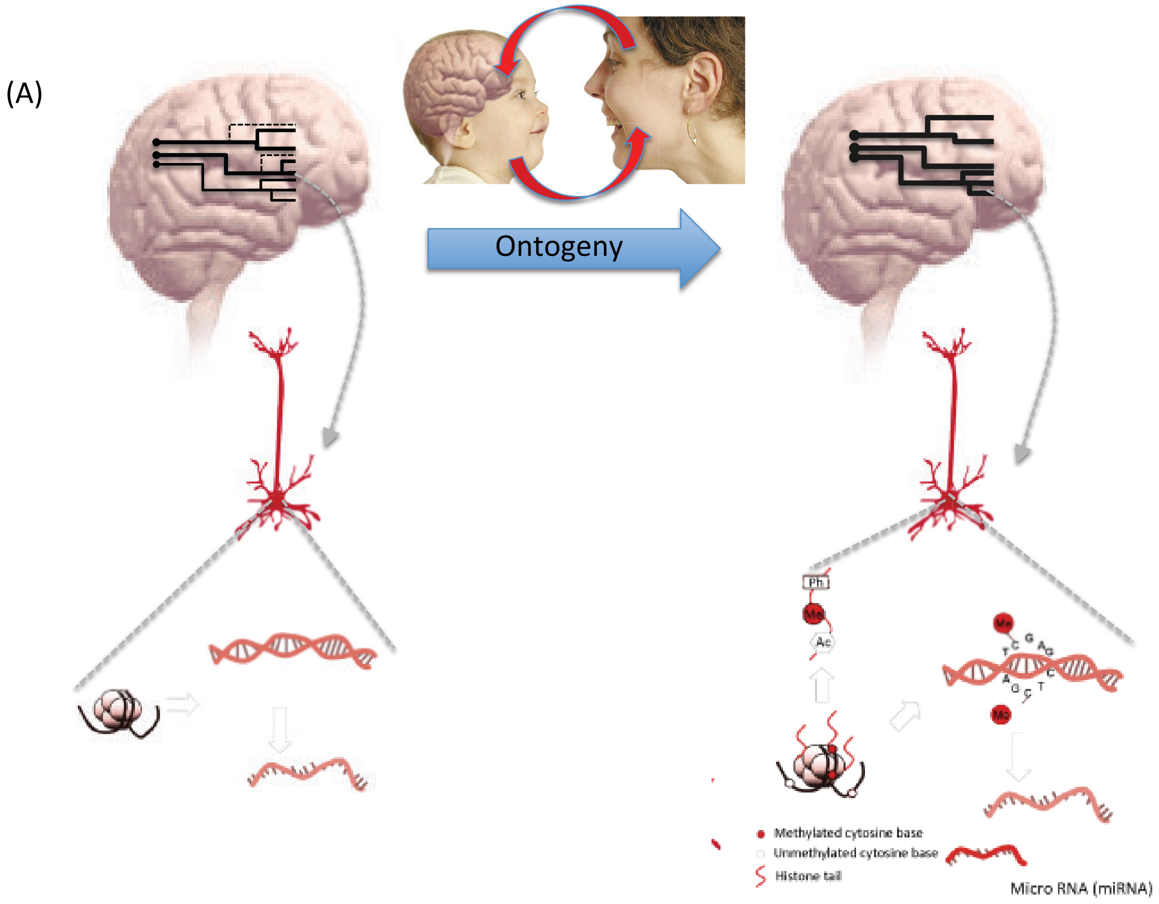
A second limitation of the *associative account* concerns mirror neurons' plasticity. The bulk of evidence in support of this account comes from work that finds sensorimotor training modulates the mirror neuron system (e.g., Catmur et al. 2008; Catmur 2013; Cavallo et al. 2013), which is interpreted as evidence that mirror neurons are not a genetically based adaptation (Heyes 2010). According to this account, if mirror neurons were an adaptation then they would not be so plastic, and, instead, would be buffered from perturbations thus showing little change as a consequence of individual sensorimotor experience or modifications of the contextual/environmental conditions (Heyes 2010). However, the evidence of mirror neurons' plasticity based on sensorimotor training is weak. First, in the key experiment supporting this interpretation, neuronal activity of mirror neurons was not directly assessed; instead, the excitability of the motor cortex was measured, which is only an indirect index of mirror neurons activity (Barchiesi and Cattaneo 2012). Moreover, recent studies replicating those by Catmur and colleagues showed that brief sensorimotor training does *not*reconfigure the mirror neuron system (Catmur et al. 2009). Additionally, the *associative account* does not consider that species-typical development of a number of fundamental genetically-based adaptations—including vision (Wiesel 1982; Wiesel and Hubel 1963; Zeigler and Bishof, 1993), human language (Dor & Jablonka, 2001), song in song birds' development (Clayton 1997), and rat copulative behaviour (Griffith and Machery, 2008) —are context-dependent, highly plastic, and significantly influenced by experience.

A final limitation of the *associative account* is that it is traditionally explored with a heavy reliance on sensorimotor training paradigms in adults, and then results, often erroneously, are extrapolated to explain processes occurring earlier in development (namely, in infancy) (Heyes 2010; Catmur 2013; Cook et al. 2014). It is true that general somatosensory experience in adulthood can cause *temporary* changes in neuronal activity without major rewiring, although, under certain circumstances, there can also be alterations of somatosensory and motor cortical maps due to increases in the strength of existing connections (Buonomano and Merzenich 1998). In contrast, experiences in infancy can cause long lasting changes in neuronal structure, particularly during critical periods of development (e.g. Leppänen and Nelson 2009; Hensch 2005; Holtmaat and Sovoboda 2009). Learning is not in and of itself sufficient to indicate that there have been significant and permanent rearrangements of connections in the brain. Moreover, during *early* phases of development (e.g. infancy), experience has different effects on the CNS. For example, work on the development of the visual system in several vertebrate species demonstrates that preserved vision in the early postnatal period is necessary for functional binocular vision through correct synaptic connections (Van Sluyters and Levitt 1980; Wiesel and Hubel 1963). Subsequent innervation may become more specific during development through the elimination of terminals from postsynaptic neurons (Holtmaat and Sovoboda 2009). These synaptic changes have long lasting effects on brain structure and function, particularly during critical periods of development, reflecting experience-expectant brain organization (e.g. Holtmaat and Sovoboda 2009). It is therefore important to distinguish these changes in brain organization in infancy from those occurring as a consequence of general experience in adulthood, in which molecular and structural elements are more stable and may, to a certain degree, impede plasticity. In other words, in adults, mature circuits are no longer capable of alternative wiring or drastic reorganization in response to typical/common environmental perturbations.

Given these serious limitations, some scientists are skeptical about the *associative account*'s ability to explain the developmental origin and function of mirror neurons (e.g. Del Giudice et al. 2009; Gallese et al. 2009; Shaw and Czekóová 2013; Rizzolatti and Arbib 1998). Alternatively, an evolutionary account of mirror neurons has been proposed, which hypothesizes that once mirror neurons emerged in development, individuals who possessed mirror neurons had a reproductive advantage, and therefore this system was retained and proliferated via natural selection (Heyes 2010).

If mirror neurons were responsible for crucial abilities for survival and reproduction, such as action understanding and imitation, mirror neurons may have become hardwired during phylogenetic history (Rizzolatti and Arbib 1998). However, this model is not without limitations. One limitation of the *genetic account* is that it hypothesizes that mirror neurons emerged during evolution such that their previous function is the same as their current function (Heyes 2010). This retained functionality, we think, is actually quite unlikely, given the common process of neural reuse, whereby neural circuits evolved for one purpose can be exapted for another purpose (Anderson 2010). In human evolution it seems that several anatomical structures and cognitive mechanisms, such as language, are exaptations, which have lost their original function (Pievani and Serrelli 2011; Fitch 2012). Instead, it seems more parsimonious that mirror neurons evolved from a mechanism that monitored the own hand goal-directed movement and were then exapted to serve additional functions, especially in humans (e.g., understand others' actions and emotional states, social learning).

An additional limitation of the *genetic account* is that it proposes that mirror neurons are present from birth, and this could be incorrectly interpreted as meaning they are purely genetically determined. We think this interpretation arises from at least two misunderstandings regarding brain development and cognitive abilities. The first misunderstanding is that, by postulating that specific mechanisms like imitation or action understanding are innate, this account fails to acknowledge that there may be critical periods during which individuals are especially sensitive or insensitive to their environments. The second misunderstanding is that this approach suggests an all-powerful conception of evolution, with natural selection processes completely shaping the development of a phenotypic trait via genetic sequences alone (e.g. mutational change)



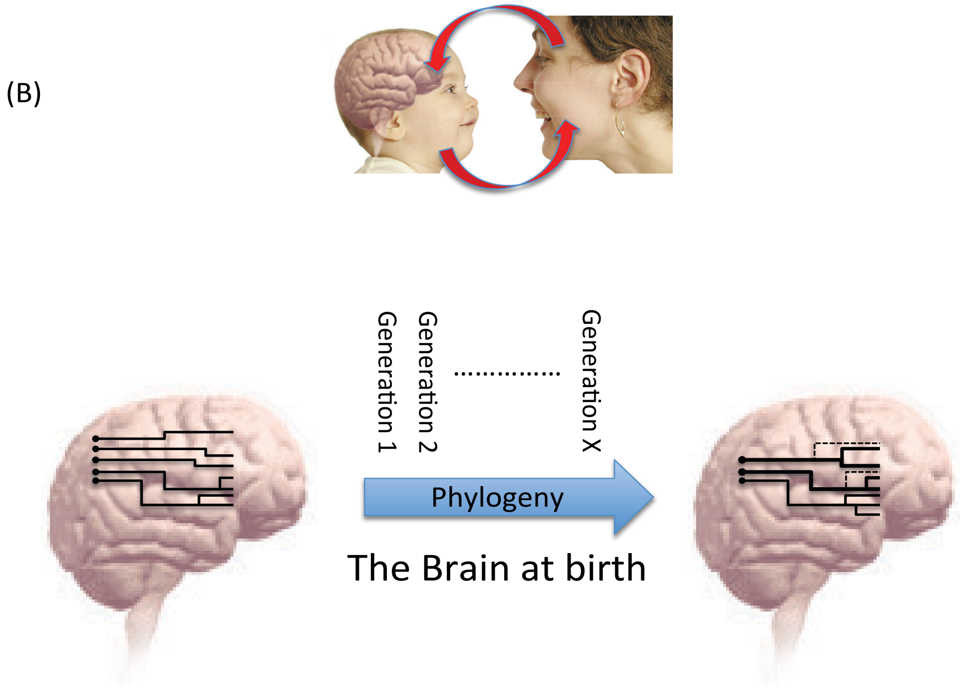


Figure 1. (A) How during ontogeny specific social experiences produce changes in gene expression. The brains on the left and right are schematic representations of potential parietal-premotor mirror circuits, which are sensitive to facial stimuli and, over the course of development, are reshaped and refined. The effective stimuli producing such changes are represented by the mother interacting with her infant through face-to-face engagement, including facial expressions. The bottom of the figure represents hypothetical changes occurring in premotor mirror neurons in the newborn brain during such social exchanges. On the left, the typical pattern of gene–protein expression in one of these neurons is depicted. On the right, the early social experience produces modifications in gene expression through epigenetic marks, such as DNA methylation, histone modifications, and micro-RNA production. Such epigenetic effects modify the pattern of neuronal wiring in the parietal premotor mirror circuits. **(B)** Hypothetical modifications that might have occurred in newborn brains if the same social environment (mothers soliciting their infants through facial expression) is present at each generation, producing a cascade of similar epigenetic events in the newborn brain. According to the epigenetic account, such plastic changes modify the neuronal wiring in the mirror circuits. The end result of these epigenetic modifications is the facilitation during the perinatal period, through yet unknown cellular and molecular modifications, of the canalization in the construction of the underlying neuronal circuits and the related developmental trajectories. Thus, the brain on the right would be, at birth, better tuned to respond to a set of social stimuli (e.g., facial expressions).

A novel proposal for the development of mirror neurons

In contrast to the accounts outlined above, we propose an *epigenetic hypothesis*, which states that mirror neurons are the result of an adaptation process involving the stabilizing selection of adaptive, environmentally-induced phenotypic traits. Unlike the *genetic account*, the *epigenetic hypothesis* supposes that mirror neurons are not the result of natural selection acting on genetic sequences that are specifically selected for the functions of goal-encoding or action understanding. In contrast to the *associative account*, the *epigenetic* *hypothesis* proposes that the development of mirror neurons is not only a process of associative learning, but also involves genetic and epigenetic phenomena, rendering phylogenetic and ontogenetic viewpoints critical for understanding mirror neuron development (*see Figure 1*).

According to this perspective, learning is central. Some authors have emphasized the importance of learning in mirror neuron development through Hebbian processes in which repeated observations of self-produced actions are coupled with motor commands to create causal sensorimotor links (Cook et al. 2013; Del Giudice et al. 2009; Heyes 2010). According to these authors, in phylogeny such learning, and the conditions necessary for producing these associations, was canalized. However, what remains unclear in these developmental models is the process or mechanism that produced this canalization, including how, and especially why this mechanism became fixed during evolutionary history. Secondly, the associative and genetic models fail to explain other important features of mirror neurons at the neurophysiological level, which are related to their variations and modulation in activity. We propose that an Evo-Devo perspective can bring such clarity, making testable predictions regarding the developmental emergence of mirror neurons and their variations that have been recorded in adult monkeys.

An Evo-Devo perspective

There is general agreement that infants at birth are attracted to specific sets of stimuli, including faces (e.g. Fantz 1963; Johnson and Morton 1991; Macchi Cassia et al. 2004; Mondloch et al. 1999; Turati et al. 2006; Valenza et al. 1996) their own hands (White 1964), and especially their own hands in motion (Van Der Meer 1997, 1995; Von Hofsten 2004), which may provide sensorimotor experiences that are the necessary scaffolding for mirror neuron development (Del Giudice et al. 2009). In the neonatal period, two important processes occur, which are relevant for mirror neuron development: Infants' neural connections between visual and motor areas are strengthened, and infants develop visuomotor coordination based on their observations of the contiguity and contingency among environmental events, such as seeing their own moving hand or synchronizing facial expressions with caregivers. It is likely that attending to sets of attractive invariant stimuli (consistently and commonly available; e.g., faces, hands) occurs from birth to develop sensorimotor control (as in the case of visually-guided hand grasping). What is peculiar about mirror neurons, however, is the *generalization process*, or the link between the perception of self-movement and the perception of others' behaviors.

Despite the fact that this generalization process is one of the most critical steps in creating the mirror and in giving mirror neurons their ‘social function,’ this process has yet to be thoroughly understood. Although speculative, we hypothesize that during the evolution of mirror neurons, visual stimuli related to others' behaviors became capable of triggering activity of a specific population of visuomotor neurons. The sensitivity of these neurons to a specific set of biological stimuli—namely, social stimuli—may be mediated, in the very early stages of brain development, by several epigenetic mechanisms involving changes in gene expression in these neurons (*see Box 1*). These epigenetic modifications were, at the beginning, not heritable but they might have produced effects at both behavioral and cognitive levels. If this new emergent neuronal response and the related epigenetic mechanisms produced some advantages to the organism (e.g., faster or more accurate capacity to recognize others' actions through mapping others' actions onto one's own motor knowledge), natural selection would have favored their stabilization and facilitation of expression under specific environmental conditions (*See figure 1b*). It is useful for the brain to be plastic early in development as this allows for the appropriate tuning of sensory motor connections into configurations appropriate for a given environment. Different developmental trajectories, thus, can be determined early in development, to help best prepare individuals for future environments. Central to this perspective is the proposal that in mirror neuron evolution, epigenetic mechanisms are sensitive to particular environmental conditions in the early stages of development. Thus, evolution supports the social and environmental conditions that contribute to specific patterns of gene expression.

As already described above, studies of neonatal imitation demonstrate a rudimentary process of visual generalization at birth (Meltzoff and Moore 1977; Ferrari et al. 2006, which is sensitive to the social environmental context (Paukner et al. 2011) and that is probably supported by a mirror mechanism (Ferrari et al. 2012). The newborn imitation phenomenon also suggests that the coupling between visual perception (of others' mouth movements) and execution (of one's own mouth movements) is facilitated in the perinatal period through yet unknown cellular and molecular modifications that are capable of canalizing underlying neuronal circuits and their developmental trajectories (*see Figure 1b*). Several researchers have investigated brain plasticity during early postnatal life, and its interaction with individual experience, at the molecular level. Interestingly, recent work in rodents has demonstrated that interactions between infants and their pre- and post-natal environments (both biotic and abiotic) are important for regulating gene expression and brain maturation, leading, in several cases, to long-lasting developmental outcomes (*see Box 1*).

Studies of epigenetic mechanisms and their stabilization in populations demonstrate that epigenetic processes may be responsible for the development and evolution of some important cognitive and emotional abilities (Fisher et al. 2007), such as stress responsiveness (Champagne 2008; Meany 2001), maternal care (Champagne 2008; Meany 2001; Rakyan and Beck 2006, and learning and memory skills (Fisher et al. 2007).

Although our knowledge of epigenetic phenomena—and particularly those involving the central nervous system—is still in its early stages, there are some examples that demonstrate the stimulus-specificity of gene expression programs (Werner et al. 2005), which may be one mechanism through which natural selection operates (Gilad et al. 2006).

This epigenetic facilitation of mirror neuron development does not consider the role of experience marginal; instead, it is often fundamental in triggering and guiding developmental trajectories. In this regard, several examples illustrate how experience might interact and shape the raw materials provided by genes. For example, the case of the callosity of skin cells in birds: even if many cells have the potential to develop callosity as consequence of pressure and friction during movement of the hind leg, only some cells present callosity at birth or soon after birth, likely the result of genetic assimilation and epigenetic mechanisms (Waddington 1975; Speybroeck 2002).

Similar epigenetic principles may be responsible for mirror neuron development, and such development appears to strictly depend on early postnatal sensorimotor experiences and social interactions. Epigenetic mechanisms underlying mirror neuron formation are yet unknown. There are certainly areas of investigation that are worth considering in future research, involving these mechanisms and their stabilization and assimilation into genetic sequences (*Box 3*). In molecular biology, some of these mechanisms are currently under investigation and scientists are making progress in understanding them (Dulac 2010; Jablonka and Raz 2009).

Another important consequence of this perspective is that it may provide insights for explaining some key visual features of mirror neuron activity, such as their modulation according to the space where the action is performed (Caggiano et al. 2011) or the type of object that is grasped (Caggiano et al. 2012; see also Casile et al. 2011 for a review). This variation in mirror neuron activity may be a consequence of the fact that, in adulthood, the environment can still exert important influences on how these neurons, and the networks in which they are connected, adjust and adapt their neuronal and functional properties to the contextual features of the environment and the individual's social experiences (*see Figure 2*).

Conclusion

The *epigenetic account* predicts that there will be variation in mirror neuron developmental paths, and for ultimately acquired properties.

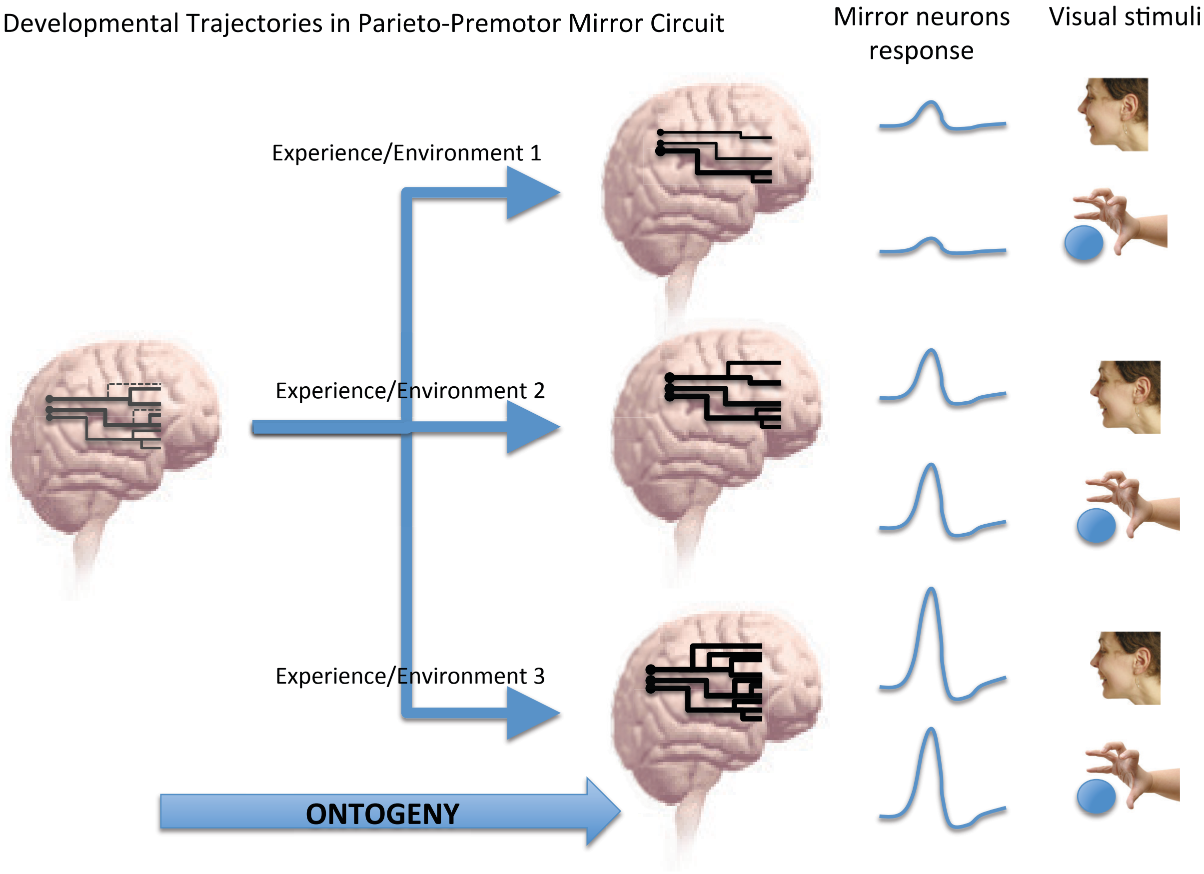


Figure 2. How different experiences might produce a variety of patterns of parietal-premotor circuits, which retain some of the basic configured features present at birth. A consequence of these changes is that mirror neurons will be produced that, in different individuals, might result in differential responses (here represented in terms of frequency of neuronal discharge) to the same set of visual stimuli (right hand side).

Though at first it may seem that the properties of mirror neurons are homogeneous, this might be due to similarities in early environments (e.g., monkeys' rearing conditions). Furthermore, this account predicts that environmental differences early in development, or under intensive sensorimotor training, should produce variations mirror neuron properties (neural response patterns) that tune them to specific stimuli related to others' actions (e.g., space or the use of tools).

Examining developmental characteristics of mirror neurons within a comparative perspective, we may consider mirror neurons to be a product of an evolved learning process, and at the same time, as a form of adaptation, having been adapted to a given set of environmental conditions (Bateson and Gluckman 2011) for which plasticity (at brain and behavioral levels) plays an important role. In this way, plasticity may be a potential adaption to unforeseen changes in environmental conditions (Bateson and Gluckman 2011).

Established after maturation, mirror neurons are not simply making a gross match of the execution and observation of self/other actions, but they are often modulated by various detailed aspects of actions (Fogassi et al. 2005; Caggiano et al. 2011; Caggiano et al. 2012; Ferrari et al. 2005), suggesting that they could be characterized not simply as fixed machinery to establish conceptual association, but as “real” variable neural circuitry subject to functional modification through the individual's history of interactions with environmental conditions. Indeed, neurophysiological research on the posterior parietal cortex (PPT) has demonstrated that areas with mirror neurons contain several other types of neurons that appear to code grasping and space in relation to actions (Rozzi et al. 2008; Hyvärinen 1982; Yokochi 2003; Fujii 2007). Thus, it is likely that this area, and the different neurons present within it, contribute to different aspects of action-perception that could be related not only to sensorimotor transformation, but also to other cognitive processes, such as space coding, and object and biological motion processing. In an evolutionary perspective, the neuronal properties of these neurons have been suitable for sensorimotor transformations, but they have been probably exapted to perform functions in other domains. The variety of neuronal properties described in the PPT highlights that sensorimotor integration is probably exploited to accomplish several functions within the physical domain (to interact with objects), as well as the social domain (to interact with other individuals).

In conclusion, an *epigenetic account* offers a powerful hypothesis to allow developmental changes in sensorimotor systems, including latent variation in mirror neuron activities, which may have contributed to niche-construction of highly sophisticated human social environments during the course of past primate evolutionary history. While genetic and associative accounts fail to consider the complexity of the interaction between genetic and non-genetic contributions, we think this new account may be utilized to solve many challenges of understanding the functional significance of mirror neurons and mirror neuron systems to subserve social interactions and, thereby provide novel insights in understanding the meanings of their disorders within an evolutionary context.

Box 1

Epigenetic mechanisms

Several researchers demonstrate the importance of epigenetic effects on development and evolution of the brain. Much work in rodents reveals that interactions between infants and their pre- and post-natal environments are important for modulating gene expression and brain maturation, leading to long-lasting phenotypic traits (Pievani and Serrelli 2011; Fitch 2012;

Anderson 2010). Such traits involve molecular phenomena (e.g., multiple post-translational modifications of histone proteins, methylation, acetylation and phosforilation, methylation on DNA), which can alter the accessibility of DNA and the density of chromatin structure in cells, such as neurons. Interestingly, some of these molecular phenomena seem to be susceptible to cross-generational transmission (White et al. 1964; Shaw and Czekóová 2013). Studies of epigenetic mechanisms and their stabilization in populations show that epigenetic processes may be responsible for the development and evolution of some important cognitive and emotional characteristics and abilities, such as stress responsively (Meany 2001), maternal care (Meany 2001; Champagne 2008) and learning and memory skills (Fagiolini et al. 2009).

Box 2

Questions for future directions of Evo-Devo hypothesis

The Evo-Devo hypothesis of mirror neurons is a useful approach for understanding fundamental phenotypic traits of organisms, in contrast to dichotomous views of the relationship between innate/acquired, adaptation/plasticity and genes/environment. This Evo-Devo view raises new questions and future directions for research to determine the mechanisms for mirror neuron evolution, such as:

- What molecular differences, at birth and during development, exist between standard visuomotor neurons and mirror neurons?
- What molecular differences exist, if any, between postnatal and adult development of mirror neurons? How do such differences affect patterns of mirror neuron discharge?
- What specific socio-environmental stimuli are able to trigger specific patterns of molecular changes underlying mirror neurons?
- When in development, if any, is there an adaptive sensitive period for mirror neurons formation? If there is a sensitive period, is it more sensitive to social-environmental, compared to non-social, stimuli? Do face mirror neurons have a developmental trajectory different from hand mirror neurons?
- What is the cognitive function of mirror neurons, beyond that operated by the visuomotor mapping?
- What are the cognitive and behavioural deficits following mirror neurons knocking out?
- What can comparisons among primates, including humans, tell us about the phylogenetic history of mirror neurons?
- What conditions have lead to the stabilization of the generalization process for creating mirror neurons, and how do these conditions vary depending on the phylogenetic history of the species presenting them?

Glossary

Adaptation is a trait that contributes to the fitness of the organism. In the traditional evolutionary perspective, the source of variation of a trait is mainly genetic and this variation is involved in the trait's expression. According to a more recent evolutionary theoretical view, (i.e., the Extended Synthesis), the stabilization of a trait within a population could occur through different processes (Dor and Jablonka 2001), e.g., hard inheritance, namely selection on genetic variations; soft inheritance, selection on non-genetic variations; (Buonomano and Merzenich 1998) and evolution of plasticity (Dor and Jablonka 2001). Adaptation, therefore, includes but does not refer exclusively to the result of a selective process acting on genes and ultimately favoring the emergence and fixation of a novel trait, which contributes to a specific function. Further, adaptations can be inherently plastic.

Adaptiveness refers to phenotypic plasticity during development; namely, the ability of a single genotype to produce more than one alternative form of morphology, physiological states, or behaviour, in response to environmental conditions. The environment (and especially the prenatal environment) is the primary source of variation in phenotypic plasticity (Leppänen and Nelson 2009). New variants of a trait can emerge as the result of developmental plasticity in which individuals differ in their response to the cellular, chemical, or social/parental environment at different stages of development. In other words, adaptiveness results from the plastic ability to overcome unforeseen environmental events (Dor and Jablonka 2001; Leppänen and Nelson 2009).

Canalization is a mechanism that narrows the range of developmental possibilities. It represents the bias that an organism has toward acquiring some forms of a trait, with a corresponding decrease in plasticity during ontogenesis (i.e., there are a greater number of developmental possibilities earlier, compared to later, in development). Canalization buffers traits against perturbation due to non-specific experiential influence and non-standard genetic variations.

Epigenetics is the study of genetic and non-genetic factors acting upon cells to selectively control gene expression. Epigenetics results in increasing phenotypic complexity during development. Epigenetic mechanisms are generally understood as chromatin modifications of genes, which allow differential access of complex of transcription factor to DNA sequences. Epigenetics also includes that study of heritable patterns of gene expression between generations, which result from methylation of DNA, chromatin structure, and genomic imprinting.

Evo – devo stands for "evolutionary developmental biology" and refers to a field of biology addressing the origin and evolution of development. It investigates the modifications of development and developmental processes that lead to the production of novel features. Three elements – epigenetics, genomic control, and environmental control – and their integration underlie and unify evolution. Epigenetic mechanisms are essential because there is no one-to-one relationship between genotype and phenotype. The genotype is the starting point and the phenotype is the endpoint of epigenetic control, while ecology is a vehicle for key innovation and integrated change during development that affects evolutionary change.

Exaptation refers to the change in function of preexisting structure during phylogenesis under appropriate condition of selection. A trait, previously shaped from natural selection for a function or alternatively resulted from a learning process, may be reused for a new function with evolutionary value.

Mirror neurons are neurons with visuomotor properties originally found in two anatomically connected cortical areas of the macaque monkeys: the ventral premotor cortex and the inferior parietal lobule. They discharge both during the execution and observation of hand/mouth goal - directed motor act and facial gesture. These neurons are not activeated by the observation of objects, or of biological movements mimicking the action, but lacking the target object. Similar neurons have been recently found also in the primary motor cortex. Some of these neurons are part of the cortico-spinal tract and may also have inhibitory discharge. The most important property of mirror neurons is the congruence they show, both in terms of goals and means to achieve the goal, between the effective observed and the effective executed action.

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Book chapter

Oxford University Press

Mirror neurons development and related evolutionary hypotheses

Prepared in 2014 and published in 2015

Antonella Tramacere, Pier F. Ferrari, Atsushi Iriki

Introduction

Mirror neurons[2] have been interpreted as an internal motor template crucially involved in social cognition: the perception of others' behavior, in fact, activates in the observer the same motor representations used during the execution of the same behavior.

Being identified by this straightforward explanation of how the brain codes others' behavior, mirror neurons have been proposed to be evolutionary conserved, although, in order to develop, they highly rely on interactions with the social environment.

Nevertheless, since phylogenetically and ontogenetically acquired phenotypic traits have been classically considered the result of distinct and opposite processes (Wimsatt 1986, relevant questions arise from these observations: how did neurons with such peculiar properties emerge in natural history? What are and to which extent do the endogenous (i.e., genetic) and exogenous (i.e., environmental) factors influence their development during the life of the organism? Further, how is the "mirror neuron system[3]" supposed to interact with other relevant brain areas, and to what extent do these interactions support additional important functions?

Three alternative hypotheses of mirror neurons' development and evolution

In recent years a debate has been sparked, for which we can synthesize three different interpretations of development and evolution of mirror neurons: the genetic, the associative, and the hybrid models (Del Giudice et al. 2009; Heyes 2010; Cook et al. 2013; Ferrari et al. 2013).

No scholars have explicitly outlined the theoretical principles embedded in the genetic hypothesis. Nevertheless, the proponents of the associative hypothesis have contributed to elaborate what was implicitly endorsed in some original papers on mirror neurons (Heyes 2010; Cook et al. 2013).

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[2] Mirror neuron (MN)—neurons with visuomotor properties originally found in two anatomically connected cortical areas of macaque monkeys: the ventral premotor cortex and the inferior parietal lobule. They discharge during the execution and observation of hand and/or mouth goal-directed motor acts and facial gestures. These neurons are not activated by the observation of objects, or by biological movements mimicking the action, but lacking the target object. Similar neurons have also been recently found in the primary motor cortex. Some of these neurons are part of the corticospinal tract and may also have inhibitory discharge. The most important property of mirror neurons is the congruence they show, both in terms of goals and means to achieve the goal, between the effective observed and the effective executed action.

[3] Mirror neuron system (MNS)—brain areas in humans that basically correspond to those in monkeys possessing MN, and show increased blood flow (detected with functional magnetic resonance imaging (fMRI)) both upon observation of others and execution of own actions. It is not completely established, because of technical limitations, if this activation was induced by identical neurons or different sets of neurons, except for the medial prefrontal cortex.

What the genetic hypothesis seems to suggest is the existence of a process of natural selection that promoted a particular set of genes and controlled the development of mirror neurons (Heyes 2010; Cook et al. 2013). It explains mirror neurons as the result of an adaptation process, during which genes have been selected in order to implement specific cognitive and behavioral functions, such as action and emotion, understanding and imitation (Heyes 2010). Accordingly, mirror neurons are conserved along evolution, because these functions have had positive effects on survival and reproduction of individuals that present them (Rizzolatti and Craghiero 2004; Bonini and Ferrari 2011).

Stating that specific genes controlling mirror neurons have been directly selected (Heyes 2010) to carry out these important functions would mean to assume a process of selection upon traits that are mainly genetically determined and to associate a specific neuronal population with one or more specific functions (Heyes 2010), falling short in the vision that makes the relation between genes, neurons, and function an univocal relationship. Yet, as molecular biology advances, this univocal relationship between genes, neurons, and functions seems extremely improbable in species with complex cognitive/motor skills and elaborated social behaviors, like primates (Wahlsten 1999; Kiberstis and Roberts 2002).

Although neurogenetic research is showing how brain structures are actually largely influenced by genetic variability, both at the population (Enard et al. 2002) and at the individual level (Thompson et al. 2001), a more complex pattern, in which multigenetic loci, genetic redundancy, and environmental control of gene expression interact with each other, is more probably involved in the emergence of specific brain areas and neuronal properties (Edelman and Gally 2001; Krakauer and Plotkin 2002), such as mirror neurons and the mirror neuron system. Therefore, instead to simply attribute the emergence and the development of specific neurons to genetic hard-wiring, an approach able to explain both the developmental robustness and variability involved in mirror neurons emergence would be more suitable and fruitful.

An additional limitation of the genetic account is that it could erroneously suggest that mirror neurons are present and fully functional from birth (Rizzolatti et al. 2002; see Ferrari et al. 2013), as if they were purely genetically determined without the possibility of the environment contributing to their tuning and rewiring in the first phases of development (Heyes 2010). Neuroscientific data are more compatible with the interpretation that the mirror system is plastic, and that neurons with a variety of mirror response properties to novel social stimuli (thus, various unique mirror neurons) can emerge during the development of the individuals (Calvo Merino et al. 2006; Catmur et al. 2009; Heyes 2010).

According to the associative hypothesis, mirror neurons are forged during sensory and motor experiences, which connects experiences of observing and executing the same actions. They emerge de novo during development, without any kind of genetic predisposition or innate information being necessary, except those underlying associative learning processes (Heyes 2010). In this case, mirror neurons are the byproduct of associative learning and they arise during development as a consequence of the limbs and body control of movements in space.

A product of learning can certainly be coopted during ontogeny to perform other functions. So, in this perspective is not in principle excluded that mirror neurons are also implicated in phenomena such as empathy, action understanding, or imitation (Brass and Heyes 2005). Nevertheless, such a position asserts that the functions that they perform have remained an epiphenomenon, and therefore have not been part of any evolutionary process, because their function has not been selected by evolutionary processes. In this perspective, mirror neurons remain a trait explainable only through the mechanism of associative learning (Heyes 2010; Cook et al. 2013).

This account, however, has some limitations. One of the most important is the "correspondence problem," which refers to how newborns link visual input of others' facial gestures to their own motor representations of the same gestures. Because infants cannot see their own face (Ferrari et al. 2013), this link appears to be present prior to any experience, making it difficult to explain human (Meltzoff and Moore 1977) and macaque (Ferrari et al. 2006) neonatal imitation from a purely associative learning perspective. In macaques, infants imitate, even in the absence of a prior experience of contingent facial interactions with caregivers, because infants in these studies are reared in a nursery from birth (Ferrari et al. 2006; Simpson et al. 2014). Furthermore, in a recent electroencephalogram experiment in newborn monkeys it has been demonstrated, that in some subjects, the mirror mechanism is present since birth (Ferrari et al. 2012).

Finally, considering the development of mirror neurons only under the control of associative learning, the associative hypothesis fails to inquire, and eventually acknowledge, that evolution may have promoted specific critical periods for the emergence of mirror neurons, during which time individuals are sensitive or insensitive to specific environmental influences.

An hybrid hypothesis has been proposed recently under the name of Hebbian learning canalization (Del Giudice et al. 2009). This hypothesis assumes, in accordance with the associative hypothesis, that visuomotor coordination during the ontogeny of the individual is responsible for the neuronal connections, which also produce mirror neurons, but adds that the molecular regulation underlying these associations were somehow stabilized and sped up along the phylogeny (Del Giudice et al. 2009). Before, a perceptual preference for organic movements, and later a simultaneous activation of motor programs and perceptual coding, induced the formation of motor neurons that encode both the execution and the observation of the same action (Del Giudice et al. 2009; Casile et al. 2011). Further, natural selection may have operated by adjusting the parameters of neonatal motor behavior and by facilitating associative learning between sensory and motor areas, resulting in the development of mirror neuron canalization (Del Giudice et al. 2009).

This hybrid model seems a plausible explanation. However, in these terms it is only a narrative version of how mirror neurons actually operate. An evolutionary account must principally attempt to answer the question of when and how a particular change happened in species phylogeny. In fact, what remains unclear in these models is the process or mechanism that produced the canalization, including why mirror neurons became canalized during evolutionary history.

Further, all the three hypotheses listed in the following sections (the genetic, the associative, and the hybrid, proposed by Del Giudice et al. 2009) do not distinguish contemporary and remote functions of mirror neurons: stating that currently mirror neurons serve action understanding does not imply that they emerged in evolution to serve the same function. In the course of development, the same structures and neural networks could be potentially involved in several other cognitive and behavioral functions, as witnessed by the processes of "exaptation" (Gould and Lewontin 1979; Gould 1991) and "neural reuse" (Anderson 2010) in the history of the brain evolution.

Finally, the three models offer an oversimplified view of mirror neurons (Ferrari et al. 2013), making difficult to explain other important neurophysiological features that are related to their variations and modulation in activity, as documented by the work of some groups in the last few years (Caggiano et al. 2009, 2013; Fogassi et al. 2005).

Development of mirror neurons by epigenetic regulation

Given all these limitations, we argue for a different hybrid model, and in particular for an account of mirror neurons through the lens of epigenetics (Ferrari et al. 2013).

In particular, in our opinion, the epigenetic approach can bring more clarity to the explanation of mirror neuron development and their recorded variations in adult monkeys and humans (Ferrari et al. 2013). Further, it can clarify how their emergence during ontogenesis could have produced novel functions that eventually resulted in evolutionary changes in the correspondent species.

In line with this approach, epigenetic mechanisms are essential because we infer there is no one-to-one relationship between genotype and phenotype, rather a complex interaction between them is likely to bring progressively different functional results. This principle, applicable to most of the neural mechanisms that undergo developmental changes, implies that the genotype of neurons in premotor and parietal cortices is the starting point to understand how, in the ontogenetic sequence, the properties of mirror neurons may emerge as the consequence of epigenetic control. In this context, social environment is one crucial vehicle for key variations and integrated changes during mirror neuron development. In order to understand such complex interactions, it is therefore important to consider the modifications that can emerge during mirror neuron development and that, from an evolutionary standpoint, could lead to the production of novel features and functions.

The epigenetic lens is used to illustrate that, at the molecular level, epigenetic phenomena are crucially involved in the emergence of mirror neurons and of the control of their functional variability. Epigenetic here is taken in a broad sense and understood as the study of the mechanisms of spatial and temporal control and regulation of gene activity during ontogenesis of the whole organism (Holliday 1990). The concept of epigenetics could then be interpreted as the etymology of the word (epi = above; gennau = genesis) as what correlates the biological origins of the organism to environmental contingencies (Waddington 1975).

From a molecular point of view, epigenetics can be synthetically understood as mechanisms of modification of DNA chromatin, such as methylation[4] and histone modifications,[5] that influence the accessibility of protein complexes for transcription of specific nucleotide sequences (Bird 2002)

Thus, in line with the associative and hybrid hypotheses previous explained, the epigenetic hypothesis of mirror neurons assumes that during neuronal development of the brain, Hebbian learning may be important in facilitating and strengthening special circuits and sensorimotor connections. In particular, visuomotor neurons develop when there is correlated, contiguous,

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[4] Methylation of DNA is a molecular process by which a methyl group (CH3) is added to the C nucleotide (cytosine), preventing the expression of the correspondent genetic sequence. The degree of methylation is passed on to daughter strand at mitosis by maintenance of DNA methylases. Accordingly, DNA methylation is thought to play an important role in the transcribable genes available at distinct cellular lineage.

[5] Modifications of histones, protein structures that are generally made up of basic, positively charged amino acids such as lysine and arginine, consist in their methylation or acetylation (addiction of acetyl group COCH3). They reduce the histone's ability to bind DNA structure, making the corresponding section of DNA available to start transcription.

and contingent, excitement of sensory and motor neurons that encode similar actions (Keyser & Perrett 2004; Del Giudice et al. 2009; Heyes 2010; Ferrari et al. 2013).

In contrast with the associative and hybrid hypotheses, the hypothesis of mirror neurons through the lens of epigenetics (Ferrari et al. 2013) presented here tries to integrate the neural and the molecular level; it proposes that, once the neural connections between the premotor, parietal, and temporal areas in monkeys have formed, mirror neurons emerge during development as a result of the process of generalization between the observation of the monkey's own movements and those of others, providing additional social functions to the individuals. The hypothesis is that epigenetic regulation underlies these processes.

We know that all neurons of the same individual contain the same genetic information and all can potentially produce the same aminoacid molecules; however, the activation and the expression of a particular set of genes play a crucial role in starting the production of specific neurotransmitters, neuromodulators, and glial cells, responsible in turn for phenotypic characteristics (domain coding) of the neurons (Greenberget al. 1986; Milbandt 1987) and, in this case, the main properties of mirror neurons.

In line with these considerations, the epigenetic hypothesis of mirror neurons suggests that the development and tuning of mirror neurons in certain areas of the brain could be accompanied by specific dynamics of a set of gene expression, via epigenetic phenomena (*see Figure 11.1*). We speculate that premotor and parietal neurons are excited during early development, by the sight or sound of actions/gestures performed by others, and that this might activate several epigenetic markers, such as methylation or demethylation of specific genetic loci, which lead to a particular pattern of neuronal activity characterizing mirror properties. The emergence of mirror neurons' activity in those areas may therefore be correlated to a population of neurons that present a particular epigenetic regulation, as result of specific social stimuli.

As also evidenced by several studies on newborns' capacity of perception and motor control towards external targets (von Hofsten 2004), the body seems to be biologically prepared to interact with specific social stimuli, such as others' body movement, or faces (van der Meer 1997; von Hofsten 2004). These early interactions between the newborn and the surrounding social environment in turn likely act on the molecular basis of neurons that, by connecting each other, may support the formation of mirror neurons and make their activity relatively stable and functional throughout the life of the individual. Thus, innate genetic programming of motor perception and stabilization of sensorimotor network through specific environmental signals act in synergy during early development.

We speculate that epigenetic modifications during the generalization process forming mirror neurons could be correlated to progressively emerging social functions in monkeys and in humans.

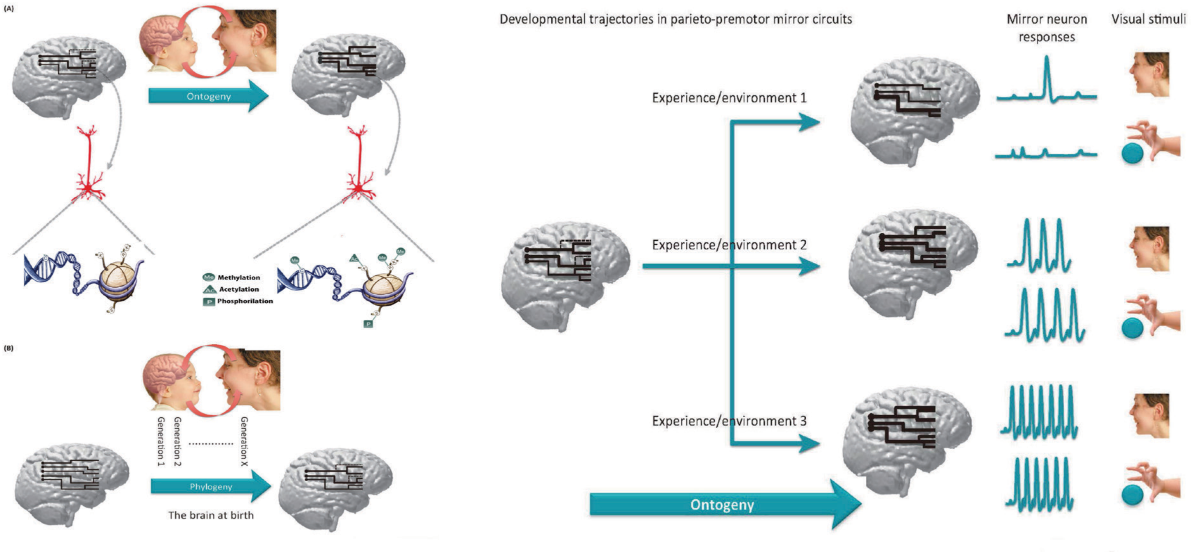

Figure 11.1 **(A)** During development of an organism, specific social experiences produce changes in gene expression. The gray brains on the left and right are, respectively, the infant and adult brain of the same individual (the baby shown in the figure). These brains show schematic representations of potential parietal-premotor mirror circuits, which are sensitive to facial stimuli and, over the course of development, are reshaped and refined. The effective stimuli producing such changes are represented by the mother interacting with her infant through face-to-face engagement, including facial expressions. On the left infant brain, a mirror neuron from the parietal-premotor circuit is shown; inside it the typical pattern of gene–protein expression is depicted. From the right adult brain of the developed infant, the early social experience produces modifications in gene expression through epigenetic markers, such as DNA methylation, histone modifications, and micro-RNA production. Such epigenetic effects modify the pattern of neuronal wiring in the parietal-premotor mirror circuits. **(B)** Hypothetical modifications that might have occurred in newborn brains if the same social environment (mothers soliciting their infants through facial expression) is present at each generation, producing a cascade of similar epigenetic events in the newborn brain. According to the epigenetic theory, such plastic changes modify the neuronal wiring in the mirror circuits. The end result of these epigenetic modifications is the facilitation during the perinatal period, through yet unknown cellular and molecular modifications, of the canalization of the underlying neuronal circuits and the related developmental trajectories. Thus, the brain on the right would be, at birth, better tuned to respond to a set of social stimuli (e.g., facial expressions). **(C)** How different experiences might produce a variety of patterns of parietal-premotor circuits, which retain some of the basic configured features present at birth. A consequence of these changes is that mirror neurons will be produced which, in different individuals, might result in differential responses (here represented in terms of frequency of neuronal discharge) to the same set of visual stimuli (right-hand side).

We suppose that both in monkey and human simultaneous activation of premotor, parietal, and sensory (audio, visual, and audiovisual) cortical areas for some basic biological movements are developmental—canalized[6] and facilitated during the very early phase of development. As for canalization we refer to the process wherein developmental pathways are

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[6] Canalization—a mechanism that narrows the range of developmental possibilities. It represents the bias that an organism has toward acquiring some forms of a trait, with a corresponding decrease in plasticity during ontogenesis (i.e., there are a greater number of developmental possibilities earlier, compared with later, in development). Canalization buffers traits against perturbation due to non-specific experiential influences.

molecularly stabilized to increase phenotypic reproducibility (Waddington 1975; Pigliucci et al. 2006). An evidence of this initial canalization comes from the presence of neonatal imitation for mouth actions both in monkeys and humans (Meltzoff and Moore 1977; Ferrari et al. 2006; Ferrari et al. 2009; Simpson et al. 2014), for which we hypothesize that during early face-to-face interactions, the visual stimuli of facial movement perceived by newborns can switch on a set of genes that are responsible for the activation of premotor and parietal neurons involved in the production of the same facial movement.

We endorse that the more plausible candidate for explaining what could have produced this early and automatic activation of a mirror response is a process of genetic assimilation[7] (Waddington 1975; Pigliucci et al. 2006) or the Baldwin effect [8] (Sznajder et al. 2012). The main idea related to these processes is that, when a learned behavior is associated with functions with high survival value, the behavior itself can affect the direction and rate of evolutionary change by natural selection (Weber and Depew 2003).

Applying both genetic assimilation or the Baldwin effect to mirror neurons encoding facial movements also implies that the sensorimotor matching between perception of others facial expression and own facial movements, at least in the postnatal phase of development, has been stablized by natural selection. In this way, both the molecular factors and the environmental conditions responsible for this imitative behavior can be inherited and facilitate its expression.

During the life span of an organism, the variable and contingent experiences faced by the individual will contribute to tune its mirror neuron system, which afterwards will have general properties, but also specific characteristics, shared with the conspecifics. As consequence, each individual motor repertoire is constrained, not only by skeletomuscular anatomy common to all the individuals of that species, but also by the acquired skills that the individual has learned in the course of his/her life (Calvo Merino et al. 2006).

Examples of this interindividual variability of mirror response in relation to the observer's specific motor expertise have been documented in several studies: by watching capoeira dancing; premotor and parietal activity is stronger in expert capoeira dancers than in dancers expert in other forms of dance (Calvo Merino et al. 2005). Differences in mirror system

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[7] Genetic assimilation is a process in which environmentally induced phenotypic traits become inherited along generations (Crispo 2007). The most plausible hypothesis for explaining what genetic assimilation is at the molecular level comes from the observation that methylation marks (H3) on the cytosine nucleotide can be transformed in thymine. Unfortunately, the process and the conditions for which this phenomenon occur remain as yet unknown (Hendrichet al. 1999; Holliday & Grigg 1993).

[8] The Baldwin effect is the hypothesis that learning accelerates genetic evolution of the phenotype. The molecular mechanisms associated with this process have been shown to be an increase along generations in allele frequency associated with the learned traits (Hinton & Nowlan 1987).

activation have been recorded between musicians and musically naïve controls when comparing observation of piano-playing movements without sound to observation of a resting hand (Haslinger et al. 2005); mirror activation differences in parietal neurons have also been found between people that had or not had a particular body trauma, like a broken leg or arm (Osborn and Derbyshire 2010). Therefore, when individuals observe actions performed by others, the motor programs recruited depend, to a certain degree, on the level and type of experience of the observer.

Epigenetic regulation of multigenetic loci is supposed to be responsible for this plastic response, as epigenetic markers are normally strong correlated with learning processes and memory storage (Levenson and Sweatt 2005; Day and Sweatt 2011).

Thus, the "basic mirror neurons system"[9], wired to a certain degree for specific actions in monkeys and humans, seems to be susceptible to "expansion," meaning the number of neurons that respond to sensorimotor training with different effectors, during actions, emotions, and somatoesthetic perception. Environmental challenges could play an important role in inducing changes in the activity of the "basic mirror neuron system," for example, by increasing the wiring (in terms of synaptogenesis and axogenesis) in mirror areas,[10] and thus facilitating the tuning to specific set of stimuli.

In line with this view, the studies on tool use and mirror neuron activity can better illustrate these processes. Tool use in macaques, in fact, recruits the same areas that are involved in hand actions, with the differences that the neurons responding to tool use are better tuned for this task after a prolonged period of visual exposure or sensorimotor training to tools (Ferrari et al. 2005; Rochat et al. 2010). Thus, tool-use neurons can be the result of a cooptation and functional shift of pre-existing hand-grasping or hand-reaching mirror neurons, and our prediction is that epigenetic processes underlying these changes in the specific brain regions.

Further, in line with this perspective, we consider the epigenetic regulation underlying the variability of mirror neurons system the field on which evolution has operated during hominid phylogeny. Thus, mirror neurons plasticity, epigenetically based, may have been the field of new evolutionary outcome in old monkey and humans (Iriki and Taoka 2012).

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[9] Basic mirror neuron system—by this term, we refer to the mirror mechanism existing in the primate individual at birth and soon after birth (although some rudimentary mirror properties may be present also in utero) activating during the perception and the execution of mouth and hand behavior. In particular, the basic mirror neuron system can be considered as mirror brain areas both in monkey and human for both executing and perceiving simple mouth and hand actions. The basic mirror neuron system for facial and manipulating actions seems to follow distinct developmental trajectory—the first one being more precocious and involved in early primate facial imitation, while the second one more late and complex. Both these mirror systems can present different degrees of robustness, while they are obviously susceptible to refinement and expansion of functions during the lifespan of organism.

[10] Mirror area (MA)—Brain areas that posses mirror neurons or show mirror responses, that compose a part of the mirror neuron system.

How has epigenetic regulation been able to act as intermediary between genes and environment, generating new variations for natural selection to operate upon? How, starting from a basic neuronal mechanism, does the epigenetic regulation operate during phylogeny, promoting the emergence of new functions?

An hypothesis of mirror neurons' evolution

Some suggestions for an answer come from the studies on tool-use in monkey.

The use of tools is clearly involved in the acquisition of high human cognitive skills and has probably played a crucial role in the evolution of human intelligence (Iriki 2006; see also Chapter 7). In contrast, the monkey use of tools is sporadic and limited to peculiar environmental factors (Iriki 2006; Iriki and Sakura 2008). As the mirror neuron system for hand manipulating actions is recruited during tool use, both in monkey (Ferrari et al. 2005; Umiltà et al. 2007) and in humans (Järveläinen et al. 2004; Rochat et al. 2010), the training given to Japanese macaques for acquiring this skill, the associated changes in neural connections, and the underlying molecular regulation, become very interesting in the analysis of how mirror neurons possibly evolved. Since humans, have evolved further in manipulating tools as compared with monkeys, then the developmental variations in the tool-user monkey's brain can give us important clues for understanding what might have been selected in mirror areas during hominid phylogeny.

As proof of the mirror neurons involvement in tool-use abilities, both premotor cortex (Area F5) and parietal cortex (PFG) are active during goal-related movements with objects such as grasping, manipulation or finger grip (Rizzolatti et al. 1988; Taira et al. 1990). Further, F5 and PFG are reciprocally connected, suggesting that this circuit, together with other parietal-premotor circuits, is devoted to the visuomotor transformation required for skilled hand–object interactions (Jannearod et al. 1995; Rizzolatti and Matelli 2003; Pani et al. 2014).

Several direct and indirect findings suggest that mirror neurons are strongly implicated in the neuronal network that control learning and performance of tool use. For example, mirror neurons and purely premotor neurons in F5 have the same motor behavior during tool using action execution (Umiltà et al. 2007). Further and more important, the existence of tool-responding mirror neurons in monkey, which in the natural environment do not use tools (Ferrari et al. 2005), suggest that this class of neurons enable the monkeys (who are normally unable to use tools) to expand their capacity to code the goal of tool-using actions by the experimenter, which do not correspond to existing action repertoires of the monkeys (Ferrari et al. 2005; Iriki 2006). In sum, all this evidence suggests that mirror neurons for hand actions are likely to be crucially involved in these skills (Iriki 2006).

Although Japanese macaques do not normally use tools in their natural habitat, two weeks of extensive training will enable these animals to use a hand-held rake to retrieve a distant food object located out of reach (Ishibashi et al. 2000). After this training, the visual receptive field of parietal neurons involved in tool use changes their response properties (Iriki et al. 1996); in fact, the image of the tool was incorporated in the body schema encoded by visuotactile neurons in the somatosensory cortex, and the receptive field that encodes the image of the hand was elongated to include the rake (Iriki and Taoka 2012). Thus, the modifications of the visual receptive fields of neurons involved in encoding learning of actions performed with tools supposed to reflect a reorganization in the modes of visuomotor integration in the parietal cortex (Iriki and Taoka 2012) and likely in premotor cortex, where mirror neurons, also in connection with other types of neurons, are implicated.

In particular, during such training, macroscopic expansion of gray matter, including the intraparietal region, was detected. In the same areas, axogenesis and synaptogenesis (Hihara et al. 2006) were accompanied with elevated expression of immediate early genes (Ishibashi et al. 1999) and of neurotrophic factors (Ishibashi et al. 2002). These findings suggest that once individuals face a novel cognitive challenge, such as recruiting sources of food with tools, mirror neurons, together with canonical and bimodal neurons, in premotor and parietal cortices (Grove and Coward 2008) undergo important structural and molecular changes (*see Figure 11.2*). The occurrence of such plastic changes during the ontogeny of brain is critical to our hypothesis, because it endorses us to inquire to what extent brain plasticity may have had evolutionary effects. Although, every biologist would agree upon the importance of brain plasticity as a fundamental adaptive trait for facing environmental changes and pressures, the question about whether, and especially how, this plasticity can stabilize novel features is still open (West-Eberhard 2003; Pigliucci and Muller 2010).

Regarding mirror neurons, the plasticity that is associated to their specialization and expansion raise the issue about the role of epigenetic regulation in their evolution. Thus, where can we find evidence of epigenetic regulation of mirror neurons and suggestions about the connection between epigenetic and their evolution? Actually, so far no epigenetic markers have been found as the basis of the plastic changes in mirror areas.

However, all the phenomena listed previously, axogenesis and synaptogenesis, elevated expression of neurotrophic factors and immediate early genes in cortical areas that may overlap with mirror system, are very likely to be underlain by complex epigenetic regulation (Bourgeois 2005; Gomez-Pinilla, et al. 2011).

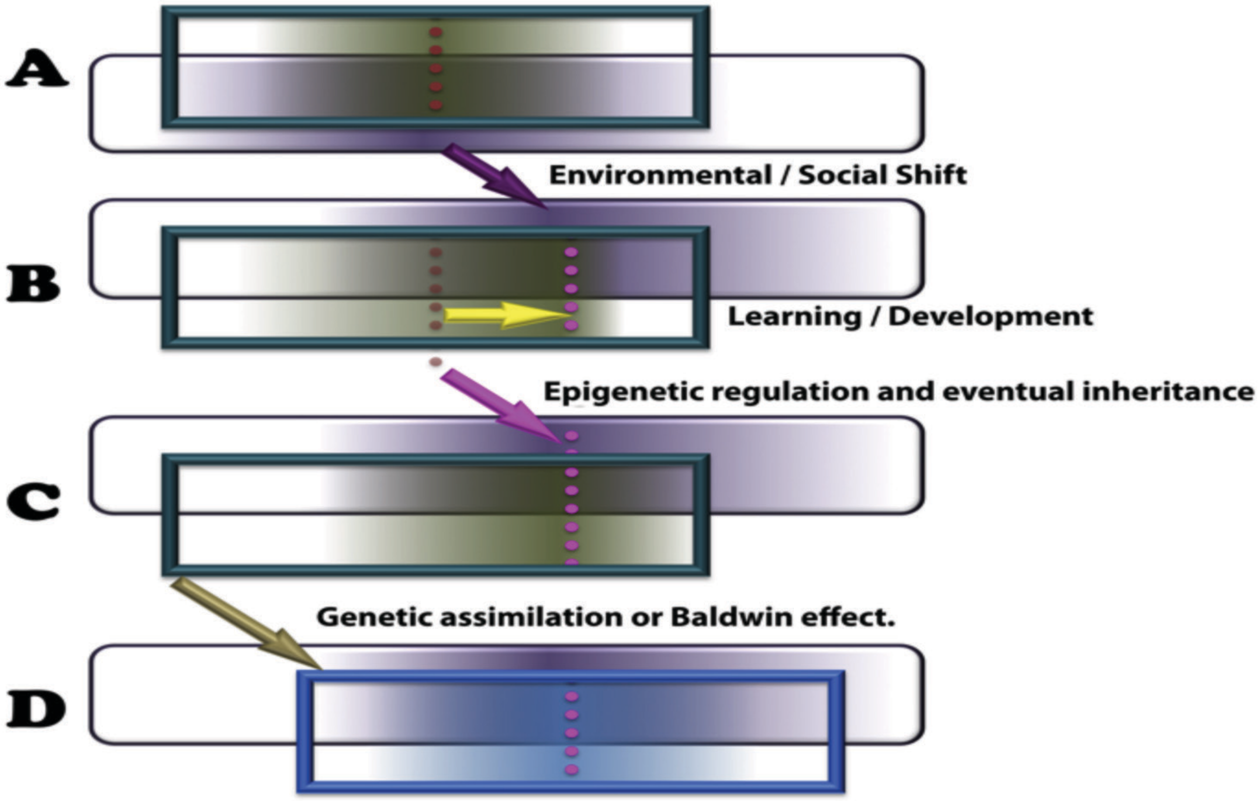

Figure 11.2 Conceptual diagram of how learning can accelerate genetic evolution of a given phenotype, passing from epigenetic regulation effects during ontogenesis to fixation through genetic assimilation or the Baldwin effect. Background squares with black outlines indicate environmental frame, whereas foreground squares with thick colored outline indicate frame of genetic sequences (different colors indicate modified sets due to genetic mutation). Shaded gradation in each frame illustrates range of variations of environmental conditions (within black frames) or phenotypic expressions (within colored frames), with respective center of variation indicated by vertical dashed line. In the original condition (A), the phenotypic expression pattern is naturally selected to match environmental variations. When environmental (including social) conditions are shifted (B, oblique purplearrow), pattern of phenotypic expression is accordingly adapted through learning (horizontal yellow arrow) within the range of plasticity mediated, by epigenetic regulation during developmental processes. When the environmental shift has been stabilized over a long period of time (C), developmental epigenetic regulation is also accordingly stabilized by natural selection and other evolutionary processes (oblique pink arrow) and inherited over generations to automatically induce a shifted pattern of phenotypic expressions, so that learning plasticity is somehow constrained. Afterwards, genetic mutations will be shifted and adapted (oblique brown arrow) to follow and optimize the center of variation of phenotypic expressions, namely genetic assimilation or the Baldwin effect.

Furthermore, recent research suggests that active epigenetic regulation, such demethylation of the Gadd45 gene family, may be one of the main molecular mechanisms responsible for the growth and differentiation of neurons in parietal cortex (Matsunaga et al. 2015).

Thus, although not specifically for mirror neurons, some evidence shows that epigenetic markers probably represent the connections between social signals and neuronal and genetic changes in mirror areas, allowing an increase in the cognitive functions of individuals.

Moreover, in the bank of intraparietal sulcus the expression of immediate early genes and the elevation of neurotrophic factors and their receptors are synchronized with the time course of the cognitive learning process, and then return to control levels once the learning process is completed (Ishibashi et al. 2002). This suggests that epigenetic regulation underlying neuronal plasticity and training-induced-genetic modulation is transient and reversible under tool-use training in laboratory conditions.

Plasticity, in fact, can be expressed at different levels, from the behavioral, to physiological, to developmental levels (Pigliucci et al. 2006). All these levels share the fundamental biological property of being part of the genotype-specific repertoire of environmentally induced phenotypes, but there are significant differences in the degree of reversibility of different kinds of the related plasticity (*see Figure 11.3*). Typically, physiological responses can be reversed over short time scales, while developmental plasticity tends to be irreversible, or takes longer to be reversed, as the latter is strictly dependent to stable changes in environmental conditions (Pigliucci et al. 2006).

In this sense, in the experiments with trained tool users, monkeys show that they possess latent cognitive abilities that can be realized by exposure to the environment; however, if the environment is not stable, evolutionary processes (such as genetic assimilation or the Baldwin effect) will not contribute to reliable stabilization of the molecular and neuronal changes necessary to express the correspondent cognitive functions. In other words, tool use gives rise to mutual interactions between the organisms and their environment, but the consequent molecular and neuronal effects cannot be stably incorporated into the brain if tools are not deeply embedded in their ecological niche (Odling Smee 2003), which is the specific environment selectively altered by organisms.

As a naturalistic example of this scenario, groups of macaques on isolated islands off the coast of Thailand have recently been discovered to be regularly using tools (Gumert et al. 2009), although this species has been long believed to be a non-tool user—and actually those on the mainland never use tools. Ethological analyses suggest that this behavior is adaptable for their immediate environmental conditions, and appears to be culturally transmitted across group members over generations (Biro et al. 2003).

Our prediction is that tool-user macaques in Thailand are experiencing, also in the mirror neuron system, the same kind of molecular, neural, and cognitive changes observed in laboratory-trained tool-user macaques.

The activities of these monkeys operating in their environment create a connection between the ecological and the neural niches,[11] intended as a portion of neural tissue adapted to accomplish a specific set of new behavioral functions, which could produce significant changes in the brain, both in terms of rewiring and in expansion (Iriki and Sakura 2008).

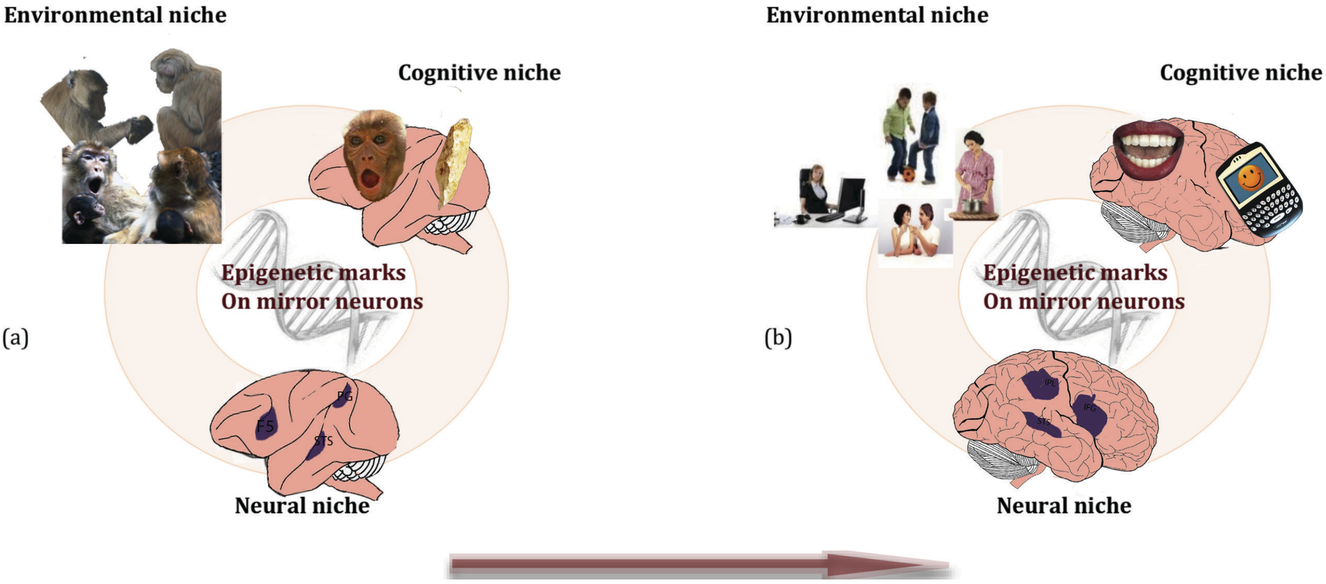

Figure 11.3 Phylogeny of mirror neurons through triadic niche construction. The orange circle represents the interactions between environmental, neural and cognitive niches. In (a) we can see these interactions in macaques, where a particular and species-specific set of contextual stimuli (environmental niche) interacts with the neural characteristics of the species (neural niche) and their functional usages (cognitive niche). These mutual interactions during development of the organism in a specific population give rise to a molecular regulation of genetic expression (also) on mirror areas. Thus, epigenetic regulation on specific genetic loci (white central circle) is at the interface between endogenous and exogenous factors producing and modifying mirror neurons.
Because of the reproducibility of epigenetic regulation and to evolutionary processes, such as genetic assimilation and the Baldwin effect, which under the proper conditions, stabilize learned traits, evolutionary shifts (red arrow) for which mirror neurons are stabilized and increased can be explained. Thus, (b) an evolved environmental niche interacts with different cognitive functions as results of a new neural endowment. New social and contingent stimuli act on the neural basis of organisms and enhance new cognitive functions, which in turn modify the environment itself. The epigenetic regulation is in this way a source of variation at the developmental level, because of which natural selection can operate.

These interdependent changes between the neural niche and the environmental niche produce the development of new cognitive properties (cognitive niche; Iriki and Taoka 2012), where

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[11] The original definition of the "niche" in biology is portions of environmental resources that collectively allow a species to persist in its habitat, namely an "ecological niche." Here, this concept of the "niche" is applied metaphorically to neural and cognitive domains. Hominin/hominid evolution has involved a continuous process of addition/integration of new kinds of cognitive capacity (cognitive niche). The dramatic expansion of the brain that accompanied additions of new portions of functional areas (neural niche) would have supported such continuous evolution. Extended cognitive brain functions would have driven rapid and drastic changes in the hominin ecological niche, which in turn demanded further brain resources (neural niche, and thereby resulting in the novel cognitive niche) to adapt to it. In this way, humans have constructed a novel niche in each of the ecological, cognitive, and neural domains, whose interactions accelerated their individual evolution through a process of triadic niche construction.

epigenetic regulation of the populational genetic heritage is the factual condition of this process.

Thus, the induction and the eventual stabilization of new cognitive functions associated to development of mirror neurons could have been achieved through a circular interaction between individuals and environments, where epigenetic regulation opened the way to new modalities of gene expression, which in turn, underlie the reorganization of neuronal connectivity between mirror areas.

This suggests to us that the development of the neural system, adapted to produce the mirror system, not only inherits a genetic code, or simply is exposed to a number of environmental stimuli and associations between them. What is inherited during successive generations is, on the one hand, the genome, and on the other the ecological niche, an environment with more technical challenges, selectively altering bodies and brains of organisms (Odling-Smee et al. 2003). This constructed environment produces specific epigenetic regulation onto gene expression and puts selection pressure on the individuals, favoring the development of traits that better match the elements present in the environment itself (Iriki and Sakura 2008), through processes like genetic assimilation or the Baldwin effect.

Conclusion

In this chapter we have proposed an account of mirror neuron development through the lens of epigenetics. Further, we have considered data regarding mirror neurons' properties and sketched out some possible evolutionary hypotheses.

From a developmental point of view, we assume that epigenetic regulation can explain both the origin and the variations found in what we call the "basic mirror neuron system" for hand and mouth actions. Once shaped, the neural connections between the temporal, premotor, and parietal areas for the production of goal-directed hand and mouth actions in primates, have, through a process of perceptual learning, allowed the same motor programs to be recruited during the perception of both own and others actions; in these the inter-individual variability of mirror responses is related to the individual's specific sensorimotor experiences.

We endorse that theory that the development and tuning of neurons with mirror properties in the brain is probably accompanied by specific regulation of genetic expression thanks to epigenetic markers, such as methylation or demethylation of genetic loci, in premotor and parietal neurons. We speculate also that the number of mirror neurons in areas of interest may therefore reflect the number of neurons with a specific methylome, here defined as the genomic distribution of methylated DNA sequences.

The connection between sensory descriptions referring to other individuals and subjective facial and limb motor representations have likely brought the mirror sensorimotor system to be involved in specific phenomena of social interaction, in which agents recognize the actions and intentions of others, making it easier to imitate communicative gestures and facial expressions. The advantages probably brought to individuals by the emergence of mirror neuron system could have led to a stabilization of the molecular and environmental conditions necessary for their emergence during the phylogenetic history of the organisms.

In particular, results from behavioral and neurophysiological research suggest that mirror neurons encoding mouth actions in the premotor cortex of macaques and humans, probably involved in imitation of facial expressions, could have been stabilized during phylogeny. The nearly automatic imitative responses of primate newborns to the affiliative gestures of the experimenter in fact lead us to hypothesize that a process of genetic assimilation (or the Baldwin effect) for which the sensorimotor matching between perception of others' facial expressions and own facial movements, became linked to the genetic sequences and rapidly expressed, despite preserving a degree of plasticity.

In contrast, mirror neurons involved in encoding hand actions may have undergone a different evolutionary trajectory. Laboratory experiments regarding tool use suggest how epigenetic regulation could have been involved, not only in the emerging of mirror neurons related to the hand, but also in their recorded variations during development. When naïve macaques are trained to use tools several changes occur in the brain, suggesting that specific brain regions involved in sensorimotor transformations have a significant degree of plasticity.

These phenomena of plasticity is very likely to be underlain by complex epigenetic regulation, in that epigenetic markers represent the connections between environmental signals and neuronal changes, allowing an increase in cognitive functions of individuals and populations. We propose that epigenetic modifications, also occurring in mirror neurons during the training process, are correlated to progressively emerging social functions in monkeys. The tool-trained macaques in fact were not only capable of proficiently using a tool, but they also showed other correlated functions, such as better imitative skills and rudimentary planning actions (Iriki 2006).

These findings strongly encourage further investigations related to mirror neurons, and in particular they suggest future experiments that can correlate neuronal properties, behavioral functions, and molecular regulation.

Moreover, these interpretations open a new and interesting scenario about the evolution of plasticity that is behaviorally induced, such as that correlated to hand mirror neurons in primates; whenever sensorimotor training produces such a profound change, increasing the complexity of cerebral substrates involved in a specific task and allowing the subjects to perform new cognitive abilities, then these changes could constitute a supplementary source of new variability in evolution.

In evolutionary terms, the manipulation of tools could have constituted a new and challenging environmental niche (*see Figure 11.2*). This new environment could have led primates to undergo profound changes in specific areas of the brain, producing a brain-derived plasticity via epigenetic phenomena. This hypothesis also requires further experimental investigation, as it is still not yet known what are the main molecular changes occurring in the mirror areas following tool use.

In sum, the account of mirror neurons through an epigenetic perspective states that neurons responding both to the observation and to execution of mouth and hand actions are partly the result of a process of stabilization of environmentally induced phenotypic traits through genetic assimilation or the Baldwin effect; this epigenetic regulation is at the interface between the genetic programming of premotor and parietal neurons and the differential developmental influences of species-specific environmental niches.

In contrast to the associative, genetic, and other hybrid accounts of mirror neurons, this model is able to explain their likely associative origin and their developmental variations in the individuals of the species presenting them. Further, it justifies a functional and structural difference in mirror system of related species of primates, proposing a distinction between proximate and remote functions of mirror neurons. At the same time, it maintains an evolutionary continuity between macaques, chimpanzee (see chapter by Hecht and Parr, this volume) and human cognitive faculties, in that correlate the cognitive effects of tool use in macaques and higher cognitive human abilities, suggesting a partial process of stabilization of the underlying mirror responses during phylogenetic history.

Acknowledgments

The work has been supported by the Laboratory of Symbolic Cognitive Development, Riken Brain Institute (Japan) and by NIH P01HD064653.

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Peer – review paper
Journal of Anthropological Sciences

Faces in the mirror
From the neuroscience of mimicry to the emergence of mentalizing

Prepared in 2015 – in press

Antonella Tramacere, Pier F. Ferrari

Abstract

In the current opinion paper, we provide a comparative perspective on specific aspects of primate empathic abilities, with particular emphasis on mirror neuron system associated to mouth/face actions and expression. Mouth and faces can be very salient communicative classes of stimuli and allow an observer to give access to the emotional and physiological content of other individuals. We thus describe pattern of activations of neural populations related to observation and execution of specific mouth actions and emotional facial expression, in some species of monkeys and in humans. Particular attention will be given to dynamics of face-to-face interactions in the early phases of development and to the differences in the anatomy of facial muscles among different classes of primates. We hypothesized that increased complexity in social environment and patterns of social development have promoted a specialization of facial musculature, of the behavioral repertoire related to production and recognition of facial emotional expression, and their neural correlates. In several primates, mirror circuits involving parietal-frontal regions, as well as insular, cingulate cortical cortices and amygdala, seem to support automatic forms of embodied empathy which probably contribute to facial mimicry and behavioural synchrony. In humans these circuits interact with specific prefrontal and temporo – parietal cortical regions, participating to higher order cognitive functions, such as cognitive empathy and mental state attribution. Our analysis thus suggest that the evolution of higher forms of empathy, such as mentalizing, is also linked to the coupling between the perceptual and motor system related to face processing, that may have undergone a process of exaptation during primate phylogeny.

Keywords: mirror neurons, facial mimicry, mentalizing, exaptation, niche construction, primate evolution.

Introduction

In the last decades, it has been an increasing number of studies inquiring the phenomenon of empathy. Originally intended like “feeling into” *(Einfühlung*) others’ people emotions and feelings (Stein 1989), empathy has been studied by social psychologists as a phenomenon causally involved in creating prosocial attitudes and behavior. From then on, empathy acquired a central importance in understanding agency in the human sciences and human beings as social and moral agents (Stanford Encyclopedia of Philosophy).

Once various levels of empathy have been investigated among different species, such as mammals and primates (Trivers 1971; de Waal 2009; Bekoff 2004), it has becoming clear that emotion understanding and prosocial attitudes are not human prerogatives, rather they have quantifiable biological basis and have been promoted in evolution, likely because of the advantages they bring at the individual and group level (Wilson 2005; Hamilton 1963; de Waal 2009).

Empathy has been defined as a multi-layered and multifaceted phenomenon, encompassing mimicry and emotional contagion at a more low – level of cognition, and sympathy, cognitive empathy and prosocial behavior at a higher level of cognitive computation *[see Table 1.Glossary]* (Preston and de Waal, 2000; Christov – Moore et al. 2014). In particular, mimicry and emotional contagion could be shared by several mammalian species, such as mice (Langford *et al*. 2006), pigs (Reimert *et al*., 2013), dolphins (Connor and Norris, 1982), elephants (Hamilton – Douglas 2006), dogs (Haidt 2001), monkey (Schwartz 2015), etc. At the contrary, the existence of higher form of empathy, such as cognitive empathy and proper altruism have been proposed only in great apes and humans (Preston and de Waal 2002).

At the cerebral level, neural mechanisms of action – perception coupling are considered a crucial correlate of at least the most basic level of empathy, such as facial mimicry and emotional contagion (Hatfield and Cacioppo, 1994; Singer 2006; Gallese 2001; Christov – Moore et al. 2014; Preston and de Waal, 2002). Such neural mechanisms have been mainly identified with the mirror neuron system, which has the key property of activating the same neuronal population during execution and perception of the same or similar actions and emotions (Gallese 2001; Iacoboni 2009). Consequently, a comparative perspective on this neural mechanism and the anatomical location in which it has been described could highlight some aspects of empathy and the factors related to its emergence in different species.

In this review, we will focus mainly on mirror mechanisms related to mouth actions and facial expression. Faces, which comprise two important regions: the eyes and mouth, are in fact very salient communicative classes of stimuli and are thought to allow an observer to give access to the emotional and physiological status of the other individuals (Darwin, 1872; Ekman et al. 1993). Mutual face recognition is crucial in fact in different social conditions, such as conflict resolution, sexual signals, parent – offspring interactions, social integration and communication (Bradbury and Vehrencamp, 1998; Regenbogen and Habel, 2015). Further, in recent decades studies on primates' mirror mechanisms related to mouth and faces have provided very interesting results, both at the developmental and comparative level (Ferrari et al. 2003; Ferrari et al. 2012; De Waal and Ferrari, 2010).

In the light of these premises, we aimed at providing a comparative perspective on primate face mirroring related in an attempt to illustrate continuity and discontinuity aspects of empathic abilities in the evolution of primates' sociality.

Mirror mechanisms in facial perception

We will describe pattern of activations of neural populations related to observation and execution of mouth actions and emotional facial expression, in some species of monkey and in human. These brain phenomena have been investigated using different brain techniques. However, the heterogeneity of the techniques doesn't allow to completely establish whether, in various brain regions of humans or monkey, identical neurons induces these types of sensorimotor matching responses. We will then specify where we can refer to mirror neurons, mirror mechanism or mirror neuron system *[see Table 1. Glossary]*.

- *Macaque*

Mirror neurons (MNs) have been localized in specific sections of premotor and parietal cortex of macaque monkeys and defined as neurons firing during both execution and perception of same or similar actions, such as for example grasping with hand or with mouth (Rizzolatti and Craghiero, 2004). Premotor MNs are connected with parietal MNs, which in turn are linked to the Superior Temporal Sulcus (STS), a multisensory area that provides the main visual input to MNs, and that possess neurons visually coding a variety of behaviors (i.e. walking, face and hand movements), thus forming what is known as the mirror neuron system (MNS) (Keysers and Perrett, 2004). Subsequently, they have been also found in medial frontal cortex, which roughly comprise pre Supplementary Motor Area (pre – SMA) and (SMA), and Primary Motor Cortex (M1) (Yoshida et al. 2011; Vigneswaran et al. 2013), thus widening the possible regions involved in the MNS.

However, in the lateral sector of the ventral premotor cortex in both right and left hemisphere, two different types of MNs have been investigated. These are involved during both the observation and the execution of actions/gestures performed with the mouth: *mouth ingestive MNs* discharge in presence of an interaction between mouth and an object, like ingestion, grasping and holding (with mouth), sucking, chewing and breaking (Ferrari et al. 2003). *Mouth communicative MNs*, in contrast, discharge in response to non – directed intransitive actions, such as lipsmaking or tongue protusion (Ferrari et al. 2003).

Lipsmaking is a typical macaque gesture related to affiliative behavior: it probably derives from a process of ritualization in which ingestive actions lost their original function to become involved in dyadic affiliative communication, thus assuming new meanings (van Hooff 1967; Ferrari et al. 2003). Lipsmacking gesture has specific features (cyclic opening-closing of the mouth) that, according to some scholars, could have played an important role in primate evolution to incorporate vocalizations in their basic motor-patterns (Partan, 2002; see also Shepherd et al. 2012; Morrill et al. 2012) making it a possible precursor of speech – like sounds (Morrill et al. 2012; Bergman 2013). *Communicative MNs* might therefore have emerged in the context of communication and could constitute a basic mechanism to map visual stimuli related to others' facial gestures into the observer's motor representation. This finding is also, somehow, intriguing since the brain control of monkey communication has been for long attributed to mesial and subcortical structure and it is thought to be involuntary, due to emotional and motivational activation (Jurgens 2002; Fogassi et al. 2013; Fogassi and Ferrari 2007). Consequently, monkeys seem to lack the discrete and voluntary vocalization that can be detected in humans and in songbirds. *Communicative MNs* can thus be interpreted as neurons implicated in the control and perception of mouth gestures as still dissociated from vocal communication in macaques. This is likely the reason for which the investigation of MNs responding to perception and execution of conditioned vocalization in macaque's premotor cortex has given negative results (Coude et al. 2011; Hage et al. 2013).

Interestingly, monkey newborns are able to imitate mouth actions, such as lips making or tongue protrusion, in the very first period of life (Ferrari et al. 2006; Ferrari et al. 2012). In neonates between days 1 to 7, in fact, perception of different facial expressions produces a plethora of facial responses that can be intended as a form of dyadic communication in the context of parent - infant interaction (Ferrari et al. 2006). This suggest that mother – infant interactions in monkey may both rely on and refine action – perception coupling mechanisms related to face and mouth.

As a matter of confirmation, specific variations in alpha frequency during EEG studies have been recorded in the context of neonatal imitation (Ferrari et al. 2012). The suppression of this rhythm, named mu rhythm, during action execution and observation, has been interpreted as the activation of sensorimotor cortex, an indirect marker of mirror neuron activity. More specifically, when adults and children view others' goal-directed actions, electroencephalography activities recorded over the motor cortex is suppressed (Marshall and Meltzoff 2011). A recent EEG study in newborn macaques has found suppression in the 5 – 6Hz frequency band in association to execution and observation of lipsmacking and tongue protrusion gestures in monkey newborns (Ferrari et al. 2012). These data are the first to show that sensorimotor structures are activated during early facial gesture observation in infant monkeys (Ferrari et al. 2012), providing important cues on the presence of a mirror mechanism at birth as correlated of infant synchronous dyadic communication.

Although pioneering, some studies are pointing the attention towards the existence of a mirror mechanism related to face coding in areas related to emotional processing, such as insula, anterior cingulate cortex (ACC) and amygdala (Gotherd et al. 2007; Livneh et al. 2012). It has been found that during both the production and monitoring of facial gestures, such as lip smacking or aggressive threat, specific sections of dorsal ACC and amygdala are activated (Gothard et al. 2007; Kuraoka et al. 2007; Livneh et al. 2012), suggesting that these regions are recruited during direct face-to-face interaction. It is thus highly plausible that the same neural populations are activated during perception of others' emotional expression related to face, as well as during the integration of the viscero – motor signals while individuals experience the same emotional state. In addition, the rostral part of insula, an olfactory and gustatory center that appears to integrate visceral sensations and the related autonomic motor responses, seems to be causally implicated in the disgust face and in facial stimuli processing and responses (Wicker et al. 2003; Kaada et al. 1949; Showers and Lauer, 1961; see also the review Gallese *et al.*, 2001). Interestingly, the electrical stimulation of this part of the insula in the monkey elicits different facial motor responses, such as disgust and lip smacking (Caruana et al. 2011). However, more studies are necessary in order to clarify the behavior of specific neural populations during emotional face perception in the insula and to trace boundary between mirror and non-mirror areas of the brain.

Nevertheless, these findings are consistent with hypothesis stating that observation of a face expression activates in the monkey a subcortical circuit involving thalamus, superior colliculus (SC), amygdala in connection with anterior insula, which may represent a fast and automatic encoding route for a rapid evaluation of facial expression (Burrows 2008). Basic visual information from SC and thalamus feeds into the amygdala, which extracts emotional cues (Haxby 2006). This subcortical pathway may then project to frontoparietal circuits through insula and ACC, implicated in the more detailed processing of facial expression (Burrows 2008). Within this pathway of face processing, some regions are also strongly implicated in motor control. Amygdala and the dorsal ACC are in fact directly connected with the facial nucleus, suggesting that they engage in the production of automatic facial movements (Livneh et al. 2012).

In conclusion, the overlapping in specific subcortical and cortical regions of different levels of action – perception control sustains the hypothesis that a mirror mechanism across this pathway are instrumental in coordinating facial display within the social context.

- *Human*

A brain circuit involving activation of specific regions of premotor, parietal and sensory cortex, related to observation and execution of similar set of mouth actions, has been discovered also in humans (Rizzolatti and Craghiero 2004; Iacoboni 2009). Through different brain techniques, mouth mirror mechanisms have been identified (Leslie et al. 2004; Buccino et al. 2001), showing that both observation and execution of different mouth configurations, such as smile or speech actions, recruited activity in ventral premotor and the posterior parietal cortex. In accordance with this result, specific sections of premotor and parietal cortices, plus part of primary motor area (M1) and pre - supplementary motor area (pre - SMA), are activated during imitation and observation of a specific set of mouth actions, such as those correlated to six main emotions (happiness, sad, angry, disgust, surprise and fear) (Carr et al. 2002). Further, MNs responding to the execution and perception of smiling actions have been recorded through extracellular recordings of neuronal ensembles, in a motor cortical area: the SMA (Mukamel et al. 2010). However, more studies at single cell levels are required to better elucidate whether other cortical motor areas of the classical mirror system have neuronal responses similar to that reported for the SMA of the monkey F5-IPL circuit.

Moreover, consistent with monkey study, amygdala, ACC and anterior insula are active during observation of emotional facial expressions (Carr et al. 2003; Wicker et al. 2003; Singer et al. 2004). In particular, anterior sector of insula is bilaterally activated when human subjects are exposed to disgusting odors or tastes. More interestingly, the insula activates anteriorly and posteriorly in relation to the observation of unpleasant and pleasant stimuli respectively, suggesting that seeing someone else's facial emotional expressions triggers the part of the neural activity typical of our own experience of the same emotion (Wicker et al. 2003). Anterior insula is not only involved in the experience and observation of disgust, but also mediate empathy for pain (Singer et al. 2004). Considering that this structure together with ACC is crucially involved in pain perception and pain-related viscero-motor reactions, it is likely that empathy for pain is mediated by a mechanism similar to that postulated for disgust (Singer et al. 2004). In addition, anterior section of amygdala is involved in processing observed fear, happy or anger facial stimuli, strengthening the conclusion that we understand the feelings of others via a mechanism of action representation shaping emotional content, so that we ground our empathic resonance in the experience of our acting body, i.e. face, and the emotions associated with specific facial movements (Carr et al. 2003; Molenberghs et al. 2012).

At the developmental level, different findings suggest that a neural system matching visual perception and executed actions maybe be active very early also in human life, and mediate dyadic interactions as primary means of non-verbal communication and intersubjectivity. Similarly to monkeys, in fact, human newborns are capable of being attentive, recognizing and reproducing at 36 h from birth, more or less six different emotional facial states expressed by the experimenter or by the caregiver (quoted by Nagy and Molnar, 2004; see also Simpson et al. 2014). Further, they tend to reproduce mouth opening and tongue protrusion in the developmental period (Meltzoff and Moore, 1983). As sensorimotor matching has been considered one of the crucial components of neonatal imitation (Meltzoff and Borton, 1979; Simpson et al. 2014), the discovery of population of neurons in parietal - frontal cortex firing during observation and execution of mouth actions has prompted the idea that a mirror mechanism may be somehow functional from birth and be involved in neonatal imitation (Ferrari et al. 2006).

Summing all these findings and interpretations, we can conclude that across investigations on monkey and human, neural mechanisms coding mouth/faces perception and execution are partly overlapping both at the neurobiological and developmental level. Although in humans anatomical studies related to brain connectivity can’t be extensively investigated as in monkeys, several findings (Paus 2005; Caria et al. 2012) suggest that both species may share a similar subcortical and cortical circuits for facial processing, opening the possibility that the two species may have solved the same biological function, facial gesture processing and coordinating face – to – face dyadic interactions, with similar anatomical substrates and neural mechanisms.

- *Emotional communication in prosimians*

In order to better understand the evolution and the functional significance of this system comparative data from other species of primates could be helpful. Neural investigations aimed to assess the existence of mirror responses have not been performed in Strepshirrine. However, since most the parietal-frontal circuits controlling hand and mouth movements have a similar patterns of connections in all primates (Kaas 2008; Preuss and Goldman – Rakic, 1989), it is plausible that also in those species, such as diurnal lemur, some forms of communications based on facial displays might rely on these circuits that operates through neural mirroring in similar ways to macaques and great apes. Although still speculative, it is possible that dyadic communicative episodes, such as for example the display of relaxed open mouth used as play signal in ring – tailed lemurs (Palagi et al. 2014), may have lead individuals of those species to couple fronto-parietal circuits for facial and mouth movements (Wu et al. 2000; Stepniewska et al. 2005) with the temporal regions devoted to face and mouth perception (Preuss and Goldman

– Rakic 2004), and probably in synergy with others subcortical regions (Burrows 2008), thus coupling the mechanisms of perception of facial gestures with the mechanisms of motor control.

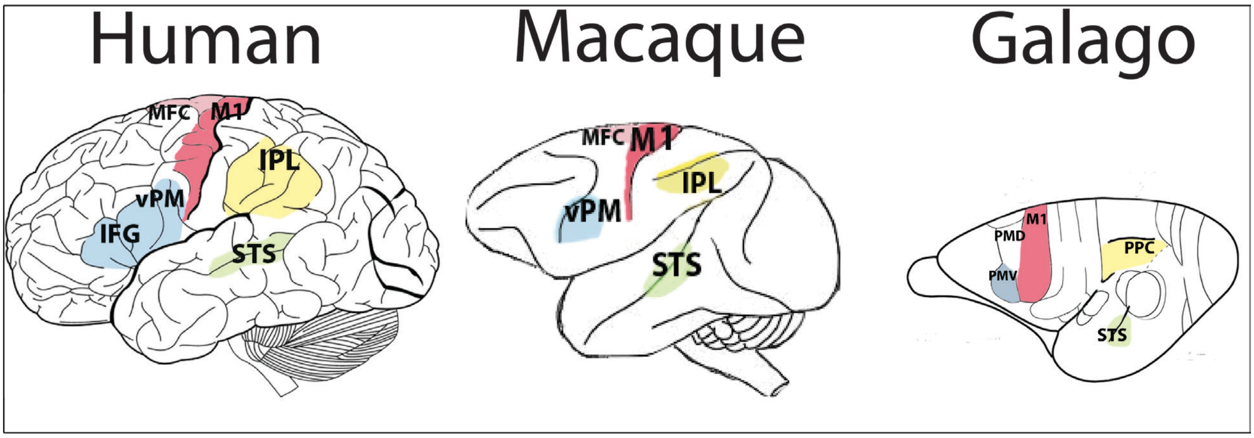
However, in lemurs complex communicative signals based on face-to-face exchanges has been observed preferentially during late adolescence and adulthood, where individuals show relatively high stereotyped frequencies of play, grooming sessions and reproductive behaviours (Doyle 1979) but not during the early postnatal period. Indeed, parents of lemurs spend very little time with their infants, who are mostly born precociously, with eyes open and ready to face the life's challenges on branches from the first weeks of development (Klopfer and Boskoff 1979). Further, given the nature of the prosimian maternal behaviour, which tends to carry babies with the mouth or on the back, the number of face–to-face interactions during mother–infant relationships seems to be very rare and less based on facial gesture exchanges (Klopfer and Boskoff 1979). These observations led some scholars to propose that prosimian infants are much less attached to their parents compared to anthropoid primates (Highly and Suomi 1986). Indeed, the existence of parental attachment is uncertain in these species and it may represent a relatively recent adaptation among primates (Suomi 1995).

Taken together these observations may have important implications on brain development, and more specifically on how cortical and subcortical networks evolved in order to sustain complex social interaction based on facial gesturing. As a matter of prediction, we suppose it might be unlikely that prosimian can mature a mouth mirror mechanism for communication in the first phases of postnatal development as it occurs in macaques. We cannot exclude, however, that they might have developed a mirror mechanism for ingestive mouth actions, since this activity is highly social and coordinated among group members. Clearly, brain analysis (fMRI or EEG) in selected prosimian species, such as diurnal lemurs, would be very useful in order to detect significant activation in specific sensorimotor regions as consequence of the observation of face and mouth actions.

Niche construction for facial mirror mechanisms

Neural mechanism coupling executed – perceived facial gestures may not be restricted to anthropoid primates. Some of prosimian species in fact experience a sufficient level of facial interactions in the adult, although quite absent in the infant phases of development, that is translated into a proper grouped organization and related intraspecific face-to-face communication (Klopfer and Boskoff 1979; Kappeler and van Shaik 2002).

Frontoparietal circuits in monkeys and humans



We show the mirror neuron system associated to facial processing in both macaque and human being. We also show a similar circuit in an adult prosimian brain, where it may be possible to find activation of same neurons during perception and execution of mouth actions and face expression.

a) **Human MNS** consists of specific sector of posterior parietal cortex, in particular the Inferior Parietal Lobule (IPL), ventral sector of premotor cortex (vPM), plus part of the inferior frontal gyrus (IFG). Mirror activities have also been reported in the primary motor area (M1), in pre and Supplementary Motor Area (pre – SMA and SMA). The superior temporal sulcus (STS) is implicated only in decoding sensory information without having been implicated in motor resonance.
b) **Macaque MNS** also involves a premotor area in ventral premotor cortex (vPM), the rostral part of posterior parietal cortex (IPL), plus part of the primary motor area (M1) and Medial Frontal Cortex (MFC). The main visual input is provided by (STS), the superior temporal sulcus, which also in macaques lacks motor properties but is considered part of the MNS.
c) **Similar temporo – parieto – frontal circuits** have been identified also in prosimians. We show a galago brain, where it is possible to find both ventral and dorsal premotor (respectively PMV and PMD), motor (M1), associative sensorial (STS) and posterior parietal (PPC) regions. MNs in these species have been not yet investigated.

It is worth to note that, in order to give a general idea of the structural similarity between the brain organizations, we depict gross activation of the cortical regions involved in mirror response during face mirroring, overlooking others limbic and subcortical areas, which participate to the mirror responses implicated in mouth/face observation and execution.

However, only with the increasing social demand during the transition to modern anthropoid primates about 40 million of years ago (Dunbar 2010), mirror mechanisms related to face expression may have become pervasive and extended to the entire life, from the very first phases of development to adulthood. Changes in social niches, such as the birth of multilevel society and more complex dynamics of parental and social bonding (Dunbar 2010) may have led to favor individuals more efficient in coordinating own facial and mouth movements in response to those of others, such as caregivers, partners, companions, and produced a stronger selective pressure on facial recognition and on the complexity of the neuroanatomical mechanisms controlling facial muscles. Indeed, Strepsirrhines clearly differ from anthropoid primates in the domain of visual communication, i.e., facial expressions and gestures. Only a few facial expressions have been reported in aggressive/fearful contexts in ring-tailed lemurs (Pereira and Kappeler 1997) and during play (play-face) in sifakas. Further, neonatal face – to – face interactions seems to be absent in prosimians (Palagi, pers. communication), while anthropoid primates present a plethora of facial signals produced primarily with a social purpose, and spend much more time in face – to – face interactions since during the neonatal phase they show the most-intricate facial displays of all mammals (Burrows 2008).

As a link between social bonding and facial expression is supported by the co-evolution of group size and facial motor control in anthropoid species (Dobson 2012; Sherwood et al. 2005), higher frequencies of face – to – face interactions may have tuned and coupled neural circuits for facial production and recognition, i.e. cortical and subcortical mirror mechanisms, and increased control of facial musculature related to eye and mouth movements.

Accordingly, the analysis of facial muscles in the three species here considered, humans, macaques and lemurs, could be highly informative. Diurnal lemurs, in continuity with nocturnal prosimians, show an intense use of olfactory signatures as a mean of individual identification, that is found to be associated to the concentration of muscle attachments around the external ear and upper lip (Burrows 2008). On the contrary, macaques, chimpanzee and humans, mostly rely on visual identification of conspecifics and they have developed more muscles in the nasolabial and eyebrow regions (Burrows 2008). Further, anthropoid primates have *depressor anguli oris* and *labii inferioris* localized around the mouth of the formers and completely absent in the latter (Diogo et al. 2009). These nerves are responsible of respectively pulling downward/inward the mouth corner and downward/laterally the lower lip (Kanade et al. 2000) and have been involved in the fine control of emotional facial expression, such as sad, happy and frown face (Waller et al. 2008).

Interestingly, in humans perception of socially relevant facial expressions (e.g. smiling) elicits in the human observer differential muscular activity in zygomaticus major muscle (Schilbach et al. 2007) that is strongly connected with the peculiar anthropoid labial muscles absent in lemur, thus strengthening the conclusion that anthropoid may have evolved a more complex *facial mimicry* as side – effect of their pervasive emotional communication face - based.

Summing up, this scenario highlights the increased role played by facial expression in anthropoids and in particular in modern humans (Burrows 2008) and we hypothesized that increased complexity in the social context have promoted a correspondent specialization of facial musculature, of the behavioral repertoire related to production and recognition of facial emotional expression and their neural correlates.

Exaptation in face mirroring

The observation that mirror mechanisms and facial mimicry can be identified during early post – natal period in human and macaques, suggest us that these species have evolved a system for pre – linguistic emotional communicative exchanges that is at work very early in life (Ferrari et al. 2006; Mancini *et al.* 2013). In this perspective, facial mimicry can be interpreted as a phenomenon that facilitates parent – infant affiliation and attachment (Mancini et al. 2013) and that is tightly linked to *emotional contagion* between individuals (Hatfield and Cacioppo, 1994). In anthropoid primate, in fact, mothers and infants often engage in intense emotional communication characterized by mutual gaze, facial expressions (e.g., smile), vocalizations (Ferrari et al. 2009), which seem to be somehow instrumental for the development of various social skills, such as goal directed behavior, emotional and intentional understanding (Steel et al. 1999; Simpson et al. 2014; Thomas et al. 2007; Belsky and Fearon 2002).

However, empathy is not limited to its fast and automatic brain/body responses. Rather, in its original meaning it encompasses the cognitive manifestation of the capacity to take perspective of another, including what others' know, want, feel or believe (Premack and Woodruff 1978; Preston and de Waal 2002). Higher form of empathy in humans are in fact interpreted as a deliberative process through which inferences can be made about others' bodily and affective states, beliefs, and intentions or *mentalizing* (Keysers and Fadiga, 2008; Zaki and Ochsner 2012). From a neurobiological perspective, in humans, medial prefrontal cortex plus temporo – parietal junction (TPJ), interacting with frontoparietal circuit engage in processing information about self and others in more abstract, evaluative terms (Mitchell et al. 2005, Uddin et al. 2005; Christov- Moore et al. 2014). Indeed, medial prefrontal cortex and TPJ are activated when human subjects attributes mental states to others, suggesting that it is implicated in theory of mind or social understanding (Iacoboni et al. 2004; Saxe 2006; Frith and Frith 1999).

Thus, if specific sectors of amygdala and insula, plus frontoparietal mirror system provide a simulative motor resonance mechanisms (Iacoboni 2009; Gallese 2001; Carr et al. 2003) connected to facial mimicry and emotional contagion, medial prefrontal cortex plus others temporal areas might be involved in self–other representations at a more cognitive/mental level, mutually interacting with frontoparietal mirror neuron system (Carmichael and Price 1995; Iriki 2006; Uddin et al. 2005).

Thus, along primate phylogeny, neural circuits with mirror properties in connection with those involved in mentalizing, might have been exapted in order to participate to more abstract cognitive functions, such as cognitive empathy. Indeed, increased activity in the anterior superior temporal gyrus and the medial prefrontal cortex are consistently reported in studies that involve some kind of social judgments such as attributing mental states and thinking about other's intentions (Castelli et al. 2002; Frith and Frith 2003; Gallagher and Frith 2004). Thus, when watching an emotional face, observer might not only participate through a synchronized mimicry, but also attribute the agent's mental state relevant for social interaction (Grosbras and Paus 2006; Shulte-Rueter et al. 2007).

It is yet under debate how the circuits implicated in mentalizing, or their connections with other functionally relevant areas, differ in monkey and human (Baron-Cohen et al. 2013). However, their crucial involvement in high-level cognitive functions open interesting experimental and conceptual perspectives in order to understand which selective pressure and mechanisms played a key role in the emergence of others' mental state attribution as connected to facial mimicry and facial expression recognition.

Conclusion

A full explanation of the complex neural and behavioral features associated with self – others recognition in the process of the emergence of empathy and mentalizing is beyond the purpose of this review. However, it seems important to us to highlight some remarks.

We hypothesize that the neonatal sensitivity of anthropoid primates to facial mimicry is strictly connected to their highly communicative environmental niche. Accordingly, it is plausible that a progressively more demanding social niche may have produced a new selective pressure on individuals that were more efficient in intraspecific communication (Schultz and Dunbar, 2007), favoring in turn the coordination of dyadic facial events also during precocious affiliative communicative situations. This new selective environment may have thus stabilized increased complexity both in facial musculature, as connected to mouth and eye movement coordination, and in related sensorimotor neural structures with mirror properties. Indeed, anthropoid primates rely more on the production and processing of facial expressions as a mechanism of close – proximity communication (Burrows 2008) and this has been associated to a general *mirroring* process, intended as a pre-conscious mechanism, which underlie and facilitate sharing of others behavior (Keysers and Fadiga 2008). In contrast, lemurs or others mammals may have evolved tools for social communication and mutual understanding based on shared odors recognition and different multisensory modality.

In humans, frontoparietal mirror circuits in connection with specific prefrontal and temporo – parietal sections of cortex, seem to be crucially implicated in others higher order cognitive functions, such as cognitive empathy and mental state attribution, suggesting that they may have undergone a process of exaptation during primate evolution. Whether changes in mate choice and parental attachment (Miller 1998), the specialization of tool – use in a social niche (Iriki and Taoka, 2008) or causes completely independent from the evolution of sociality (Shea 1989) gave rise to the pronounced cognitive empathy in humans is yet unknown. However, this analysis suggest that the evolution of higher cognitive skills, such as mentalizing, is also linked to the coupling between the perceptual and motor system related to face processing and that involve affective and emotional processes to a greater extant than previously theorized.

Glossary

Mirror neurons have been firstly discovered in macaque monkeys and defined as neurons firing during both execution and perception of same or similar actions, such as for example grasping with hand or with mouth. They have been localized through single cell recording in the premotor cortex (F5), and in the Intraparietal Lobule (IPL). Furthermore, a recent experiment suggests that they might be present also in primary motor cortex, an area strongly connected with premotor regions.

Mirror neuron system is a network of interconnected area that simultaneously process information about mouth and hand actions execution and observation, comprising the superior temporal sulcus (STS), containing no mirror neurons, and sections of premotor (F5) and parietal cortex (IPL), Medial Frontal (MFC) and Primary Motor Cortex (M1).

Mirror mechanism is a brain mechanism, measured often by fMRI and EEG, of action-perception and is often used to describe the property of the brain (within the traditional mirror areas but also outside them) to have same/similar pattern of activity during observation and execution of action.

Facial mimicry is an involuntary, rapid and automatic response in which an individual mimic the facial expression of another individual. This phenomenon can be distinguished from other voluntary and cognitive form of imitation, because of the rapidity of the response involving exclusively the face.

Emotional contagion is the transfer or communication of a certain mood among individuals. It can also be defined as the tendency for two individuals to emotionally converge. This can verify through automatic mimicry, synchronization of facial or gestural expression, vocalization or postures synchronization.

Cognitive empathy or mentalizing is often defined as “perspective taking” and it refers to the ability to understand through inferential evaluative processes the emotions, feelings, desires or beliefs of other individuals.

Acknowledgments

The work has been supported by NIH P01HD064653.

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Peer – review paper

Mirror neurons in the tree of life
Mosaic evolution, plasticity and exaptation of sensorimotor matching responses

Prepared in 2014-2015 – under submission

Antonella Tramacere, Telmo Pievani, Pier F. Ferrari

Abstract

Considering the properties of mirror neurons across development and phylogeny, we offer a novel, unifying, and testable account of mirror neuron evolution and unify a substantial amount of apparently discordant research, including mirror neurons plasticity during development, their adaptive value and their phylogenetic continuity. We hypothesize that the mirror neuron system reflects a set of interrelated traits, each with an independent natural history due to unique selective pressures, and propose that there are at least three evolutionary significant trends that gave raise to three subtypes of mirror neurons: hand visuo-motor, mouth visuo-motor, and audio-vocal. Specifically, we put forward a mosaic evolution hypothesis, which posits that different types of mirror neurons may have evolved at different rates within and among species. Finally, the manuscript offers a strong heuristic potential in predicting the circumstances under which specific variations and properties of MNs are expected. Such *predictive value* is critical to test new hypotheses about MNs activity and its plastic changes, depending on the species, the neuroanatomical substrates, and the ecological niche.

Keywords

Exaptation, homology, neonatal imitation, manual gestures, mirror neurons, mosaic evolution, plasticity, sensorimotor learning, tool–use.

Introduction

Among sensorimotor neurons, a subclass of neurons fires both when an individual performs an action and observe that same action performed by another. These neurons, called mirror neurons (MNs), were first described in the ventral premotor cortex and inferior parietal lobule of macaque monkey (Di Pellegrino et al. 1992; Gallese and Goldman 1998; Rizzolatti and Craighero 2004). MNs gained popularity (Ramachandran 2000; Heyes 2010): the matching between perception and action at the level of single neurons, was deemed relevant to several fields of research and MNs have been proposed to play a key role in social cognition (Gallese and Goldman 1998; Rizzolatti and Craighero 2004).

Research on MNs has paved the way for the formulation of various hypotheses based on interpretations of their (i) possible function, (ii) mechanism, (iii) ontogenetic development and (iv) evolutionary history (Rizzolatti and Craighero 2004; Rizzolatti and Arbib 1998; Bonini and Ferrari 2011; Cook et al. 2014). The present article however is concerned with a comparative analysis of MNs that considers properties and factors associated to MNs development in different species.

Along this line, we will mostly consider MNs development and phylogeny. Nevertheless, topics related to functions and mechanisms will be covered as well. Indeed, although these four questions (development, evolution, function and mechanism) refer to different level of explanations (Tinbergen 1963), they are not completely independent from each other and they should be also analyzed in order to fully understand the evolution of MNs as a functional trait. To use a metaphor, consider the Rubik's cube: every attempt to set a cube's colored faces to match on one side implies a new arrangement of the other side's faces. Similarly, each different answer to one of the questions entails at least a partly different answer to the other questions.

Briefly, some neuroscientists propose that macaque and human MNs are an evolutionary conserved neural mechanism that has been selected during phylogeny for accomplishing high-level cognitive functions, such as action understanding, imitation, mind-reading and language (Rizzolatti and Arbib 1998; Gallese and Goldman 1998; Gentilucci and Corballis 2006). These views moslty focused on the functional role of MNs during phylogeny and somehow neglected the developmental processes that contribute to its construction during ontogeny (Keysers and Gazzola, 2014).

On the contrary, some models mainly addressed the question of the ontogenetic origin of MNs. According to an associative model (Cook et al. 2014), MNs acquire their observation-execution matching properties through a domain-general process of sensorimotor associative learning and have no significant evolutionary function (Catmur et al. 2007; Heyes, 2010; Cook et al. 2014). A complementary Hebbian learning model (Keyser and Perrett, 2004; Keysers and Gazzola 2014) focus more on the spike-time-dependent plasticity that occurs at the synapses and the anatomical details of the connections giving rise to mirror neuron system. It proposes that Hebbian learning (i.e., synaptic efficiency through concurrent neural firing) plays a major role in wiring together sensory and motor areas of the brain and subsequently generalizing them to actions performed by others.

Alternative models have proposed an integration of MNs' developmental and evolutionary dynamics, focusing attention on the role of regulative factors underlying developmental plasticity (Ferrari et al. 2013) and canalization processes (Giudice et al. 2009; Ferrari et al. 2013). These hypotheses consider MNs as the result of epigenetic regulation of specific populations of motor neurons under the influence of particular social experiences during ontogeny (Ferrari et al. 2013; Tramacere et al. in press). It also proposes that some of the environmental, social and molecular conditions, which contribute to the development of MNs, have been canalized or stabilized during phylogeny, promoting the adaptive ability to decode social information and facilitating social interactions from the first phases of development (Giudice et al. 2009; Ferrari et al. 2013).

While associative accounts (Heyes, 2010; Cook et al. 2014) have denied the possibility that MNs have a specific functional role and that they could have emerged through an evolutionary process as specific adaptations, other scholars have argued that the phylogeny of MNs relates to the evolution of highly specialised (and often 'higher') cognitive faculties, such as language (i.e. Rizzolatti and Arbib 1998) or mind-reading (Gallese and Goldman 1998). In contrast to both these views, the alternative models influenced by research in epigenetics (Giudice et al. 2009; Ferrari et al. 2013) have sought to provide a speculative narrative of how mirror neurons evolved. However, while an evolutionary account must principally attempt to answer questions of when and how a particular change happened in species phylogeny, in these models what remains unclear is the process or mechanism that produced the canalization, including why mirror neurons became canalized during evolutionary history. In this paper, we try to bridge these gaps. In addition, while previous models are based on information derived from studies on macaque and human MNs, we expand our perspective and review by including additional critical information regarding MNs in songbirds (Prather et al. 2008) and marmosets (Suzuki et al. 2014), as well as inferences based on careful analysis of brain activity through neuroimaging in another primate species, the chimpanzee (Hecht et al. 2013).

In light of these new data, we formulate a new view of MN evolution, consistent with comparative neuroanatomical and behavioral evidence to date. Further, we suggest some directions for future research in the analysis of sensorimotor neural structures.

Are Mirror Neurons a valid trait to compare across species?

In order to analyze MNs from an evolutionary perspective, it is necessary to identify them as a valid trait to compare across species *[see Table].* A valid trait is identifiable by reliably being present in many individuals and distinguished from other traits (Striedter 1999). In the nervous system, a valid trait, such as a brain region, is defined in terms of specific attributes, including location, cytoarchitecture, physiology, pattern of connections and functions (Kaas et al. 1983; Tyner 1975; Striedter 1998; Striedter 1999). We will discuss these attributes below.

MNs have been investigated in four species of macaque monkeys, and localized in specific sections of the ventral premotor and parietal cortex (Di Pellegrino et al. 1992; Rizzolatti et al. 1996). Furthermore, recent experiments suggest that MNs might be present also in medial frontal cortex (Yoshida et al. 2011) and primary motor cortex (Vigneswaran et al. 2013), an area strongly connected with premotor regions.

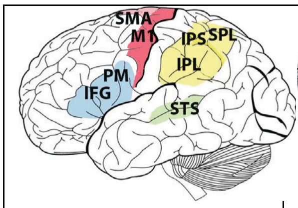
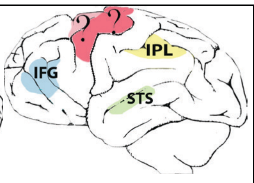
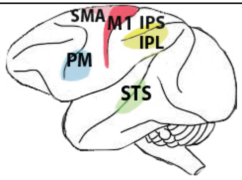
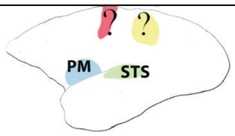
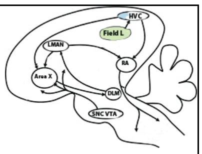
In humans, MN activity has been indirectly inferred in specific sectors of premotor, motor cortex and inferior parietal lobule (IPL), plus the intraparietal sulcus (Fadiga, 1995; Hari, 1998; Oberman, 2005; Iacoboni 2009; Tunik et al. 2007). These data have been also confirmed by at least two meta-analysis studies: across 139 fMRI and PET experiments (Caspers, Zilles, Laird, and Eickhoff, 2010) and another 76 fMRI studies (Molenberghs, Cunnington, and Mattingley, 2012), the inferior frontal gyrus, ventral premotor cortex and inferior parietal lobe were active to both the execution and observation of body actions. Mirror activity at the level of single neurons was also investigated in few studies in which neurons with mirror like properties have been reported in the Supplementary Motor Cortex (SMA) (Mukamel et al. 2010), and in the anterior cingulate cortex (ACC) (Hutchinson et al. 1999), although in this latter study only a single observation was reported. Finally, mirror responses in parts of human limbic system were confirmed through functional magnetic resonance imaging (fMRI) in specific sectors of the amygdala and the anterior insula (Carr et al. 2003, Wicker et al. 2003) as well as other brain structures implicated in somatosensory perception, like the secondary somatosensory cortex (SII) (Keysers et al. 2004; Osborn and Derbyshire, 2010; Ebisch et al. 2014).

Furthermore, recent studies employing PET imaging, found mirror-like activation in the frontal and parietal cortex in chimpanzees (Hecht et al. 2013). Interestingly, a recent study reported neurons discharging during execution and perception of the same actions in a sector of the premotor cortex of marmosets (Suzuki et al. 2014).

Neurons with mirror properties have not been restricted to the primate order, but extend to other taxa as well. In songbirds, MNs for vocalization have been localized through single-cell recording in the HVC (High Vocal Center) song nucleus, a premotor area necessary for learning and for voluntary control of songs (Prather et al. 2008).

As evident in *Table 1*, we excluded from our analysis MNs activated by the observation of emotional facial expressions and those activated during pain-related stimuli, resulting in the activation of areas of the limbic system, such as amygdala, anterior insula, anterior cingulate cortex and secondary somatosensory cortex (van der Gaag et al. 2007). These MNs have been investigated primarily in humans (Carr et al. 2003; Wicker et al. 2003) and therefore, we have, to date, insufficient evidence across species to speculate about their evolution.

Table. Mirror neurons across species

				
Homo sapiens	*Pan troglotydes*	*Macaca* - *nemestrina* - *mulatta* - *fascicularia* - *fuscata*	*Callitris jaccus*	*Swamp sparrow* *Bengalese finch*
fMRI PET TMS MEG EEG single - cell	PET	fMRI EEG single – cell	single – cell	single – cell
Supplemental Motor Area (SMA), Primary Motor Cortex (M1), Superior Temporal Sulcus (STS), Inferior Frontal Gyrus (IFG), Inferior Parietal Lobule (IPL), Premotor Cortex (PM), Intraparietal Sulcus (IPS), Superior Parietal Lobule (SPL)	Inferior Parietal Lobule (IPL), Superior Temporal Sulcus (STS), Inferior Frontal Gyrus (IFG)	Supplemental Motor Area (SMA), Primary Motor Cortex (M1), Inferior Parietal Lobule (IPL), Intraparietal Sulcus (IPS), Premotor Cortex (PM)	Premotor Cortex (PM), Superior Temporal Sulcus (STS)	HVC (High Vocal Center) Field L

While MNs are defined as neurons that *fire during perception and execution of the same action* it is important to note that, because of technical limitations, we cannot be completely confident that in humans or chimpanzees single neurons or populations of neurons induce these types of sensorimotor matching responses. Nevertheless, some studies (Nelissen et al. 2005; Nelissen et al. 2011) performed with fMRI in awake macaques have shown increased activation upon observation of grasping actions in areas where macaque MNs have been recorded through single cell studies, suggesting that fMRI may disclose cortical areas with mirror properties, and it is therefore plausible that single neurons with mirror properties are present in the frontoparietal circuits of humans too. Moreover, electrode recordings in humans' motor cortex revealed observation-execution matching responses at the level of single neurons (Hutchinson 1999; Mukamel et al. 2010).

Thus, here we define MNs as discrete *populations* of neuronal cells active during the perception and execution of actions/gestures. The definition of MNs at the population level implies that the activation found through brain imaging studies in macaques, chimps and humans likely reflects the activity of populations of neurons that are similar to those described at a single cell level in monkeys. MNs seem to retain also a very similar pattern of connectivity in primate species (Keyser and Perrett 2004; Hecht et al. 2013). They have been in fact considered as being embedded in the mirror neuron system (MNS), a network of interconnected areas that simultaneously process information about biological actions, including their execution and observation (Rizzolatti and Craghiero, 2004; Keyser and Perrett, 2004), comprising the superior temporal sulcus (STS) that contains no mirror neurons, and specific sections of premotor, parietal and motor cortex that do contain MNs *[the connectivity of songbird MNs will be considered in 2.3 section].*

In monkeys and humans, MNS have been hypothesized to be involved in action recognition. Being recruited during visual perception, MNs could, in fact, allow the individual to produce a motor representation of what another individual is doing (Rizzolatti and Craghiero, 2004), activating an internal description of various attributes (i.e. action direction, action goal, spatial location, kinematics) relevant to action execution (Rizzolatti et al. 2001).

Consistent with this functional attribution to MNs, a recent study performed through transcranial magnetic stimulation (TMS) in humans highlighted the causal role of population of premotor neurons in recognition task through observation of lip and hand actions (Michael et al. 2014). A meta-analysis of 11 studies that involved more than 350 patients with brain lesions in inferior frontal cortex and the posterior parietal cortex found impairments in the capacity of patients to recognize others' actions, thus further supporting the casual role of the temporo-parietal-premotor brain network in action recognition (Urgesi et al. 2014). Further researches however are needed to corroborate this hypothesis and to find parallels in non-human primates.

From a cytoarchitectonic perspective, MNs seem not to be linked to specific properties of the cerebral cortex (granular, agranular or dysgranular). For example, human MNs have been localized in the premotor area (BA6), which is agranular, and in Broca's area, constituted by BA 44 and BA 45, which are respectively dysgranular and granular (Brodmann, 1909; Amunts et al. 1999). The same combination of cytoarchitectonic properties has been found in macaque and chimpanzee premotor and parietal mirror areas (Hines, 1929; Luppino et al. 1993). However, further investigations are needed to verify whether the neurophysiological properties of MNs are linked to a specific cortical layer, such as layer III, which contains both agranular and dysgranular tissues (Shipp, 2005; Shipp, 2007).

It is worth noting that characters or traits in comparative biology are not defined by any essential properties. Rather a trait must satisfy as many attributes (i.e. location, physiology, functions, pattern of connections, cytoarchitecture) as possible (Tyner, 1975; Striedter, 1999). Thus, the lack of one of these attributes is not a sufficient reason to dismiss the validity of the trait, nor the possibility of a homological relationship between traits (Tyner, 1975; Striedter, 1999).

Different categories of Mirror Neurons

Although MNs are a defined and recognizable trait sharing a core neural matching mechanism of action – perception, to be compared across the amniotes[i], a closer inspection suggests that MNs in such heterogeneous taxa do not reflect a uniform and stable execution-observation matching system both within and across the species (Ferrari et al. 2013). Firstly, they can be activated in different sensorial modalities, like during vision (Di Pellegrino et al. 1992), hearing (Pulvermüller et al. 2006), or both (Kohler et al. 2002), and can involve different effectors, like hand (Rizzolatti et al. 2001), mouth (Ferrari et al. 2003), or, in the limbic system, by a combination of them (Gallese et al. 2004; Keyser & Perrett, 2004). Secondly, MNs are highly plastic and highly variable in their locations and proximate functions during ontogeny (Calvo Merino et al. 2005; Haslinger et al. 2005; Calvo Merino et al. 2006).

In order to deal with such variability and complexity, we propose a simple and preliminary distinction that may help to clarify MNs evolutionary history by taking into account their various dynamics of sensorimotor processing. We will classify MNs using two unambiguous physiological criteria: the modalities of sensory input triggering the response, and the effectors involved in the motor output. With this approach, we obtain three main categories of MNs: *hand visuomotor MNs*, *mouth visuomotor MNs*, and *audio–vocal MNs*. We will then address the question of the evolutionary relationship for each category.

Hand MNs in primates: a common evolutionary history

With *hand visuo-motor mirror neurons* (hand MNs) we refer to a population of neuronal cells activated by the visual observation of others' hand gestures, and also involved in the control of own hand actions *[See fig. 1]*.

Located in the frontoparietal circuit of macaque, hand MNs respond to the observation and execution of grasping actions (Rizzolatti and Craghiero, 2004). The response can, in some cases, be specific for the type of grip (e.g., precision *vs.* power grip) or for the type of actions (i.e., manipulation, holding etc) (Rizzolatti et al. 2002). In other cases, MNs may exhibit a large degree of generalization, firing both in response to actions performed by conspecifics as well as those by heterospecifics (humans), even when these are performed from different visual perspectives (Caggiano et al. 2009). Further, neuroimaging studies show that intransitive or mimicking actions elicit very weak activation in mirror cortical regions (Nelissen et al. 2011).

Furthermore, mirror responses have been found in the frontoparietal cortex of chimpanzees during the execution of grasping actions, observation of transitive grasping actions and intransitive movements (Hecht et al. 2013). Chimpanzees have a MNS, supported by the same cortical regions as those consistently found in humans, namely the premotor and parietal areas as well as the STS region. Interestingly, and in contrast with macaques, chimpanzees show similar motor activation also during the observation of non-goal directed actions (i.e. miming grasping), thus resembling the properties of the human mirror system (Hecht et al. 2013). In humans, both intransitive and transitive gestures enable the activation of the mirror system (Rizzolatti et al. 2002).

Mirror responses have also been found in the ventral portion of the frontal cortex of marmosets, during the execution and observation of transitive reaching and grasping actions (Suzuki et al. 2014), widening the range of species that show visually activated motor neurons and suggesting that, in primate phylogeny, MNs are probably evolutionary more ancient than previously thought.

The recruitment of hand visuomotor mirror responses during visual perception may be involved, in terms of proximate causes, in the function of *action recognition* in several social species. A similar mechanism could have had an evolutionary role during primate phylogeny. Another possible function of MNs, which has been put forward some years ago by Jeannerod (1994), is that their property seems to be suitable for imitative purposes. Neuroimaging studies in humans has confirmed that the core areas of the MNS are activated during both the simple imitation of mouth gestures and hand movements not directed towards a target (Iacoboni et al. 1999; Carr et al. 2003; Iacoboni et al. 2005). Interestingly, such results seem to contrast with the monkey studies. Functional MRI investigations show in fact that in monkeys the MNS respond very weakly during the observation of non goal directed actions, while in chimpanzee and humans the mirror system is also activated by intransitive, meaningless movements. Furthermore, no monkey studies has investigated the possible role of MN during imitation because macaques are considered to have poor imitative capacities, and even through training, the main route of new motor skills acquisition can be hardly through an imitation process. According to this explanation, the monkey mirror system would appear to be sensitive to the goal of actions, but not to code the details of the observed action that lead to the goal. This may be a possible explanation of the behavioral evidence that monkey cannot replicate the observed actions, although they seem to recognize goal directed movements during perception (Rizzolatti, 2005; Rochat et al. 2008). These observations raise the possibilities that in primate phylogeny, the activation of MNs by intransitive and meaningless actions might be the result of recent adaptive evolution that led to an autopomorphic (taxon-unique derived trait) of the mirror machinery in hominids (chimps and humans).

Support to the hypothesis that stronger manual imitative abilities in humans and chimpanzees, compared to macaques, appear to be related to species differences in features of their MN systems comes from anatomical studies as well (Hecht et al. 2012). From a behavioral point of view, macaques only copy or emulate the end-result of observed hand actions, while humans and chimpanzees copy the step-by-step action process (Huffman and Quiatt, 1986; Visalberghi and Fragazy, 2002), even though between humans and chimpanzees there exist several differences in the way they imitate (Tennie et al. 2012; Friedland and Moore, 2014). This distinction is likely reflected in the evidence that macaques do not acquire tool – use by imitation learning, while chimpanzees and human do (Biro et al. 2003; Whiten et al. 2005). The explanation may rely on differences in neural connections through which MNS is embedded (see Hecht et al. 2012). In macaque there is a large discrepancy between the ventral (superior temporal sulcus with frontal areas) and the dorsal circuits (parietal lobe with frontal areas), linking the main sources of visual information related to biological meaningful stimuli (i.e. hand/body movement, face perception, gaze movement) to cortical areas involved in higher cognitive functions. In general, the ventral connections much larger and strengthened than the dorsal ones; this difference is less pronounced in chimpanzees and absent in humans (Hecht et al. 2012). Functionally speaking, the ventral route may be useful in coding the physical end-result of observed actions, while the dorsal route may code the spatial mapping of movements and may extract finer level of action kinematics (Johnson-Frey et al. 2003; Goldenberg 2009; Hecht et al. 2012).

Moreover, in humans, but not in other species, an additional dorsal pathway passes through the parietal opercular white matter to the anterior supramarginal gyrus, and this pathway seems to be implicated in tool-use in humans (Iriki 2006; Peeters et al. 2009; Hecht et al. 2012). Finally, the link between the mirror parietal region (enlarged in human and associated with spatial awareness) and the inferior temporal sulcus, where objects and tools are recognized, is strongest in human, intermediate in chimpanzee and weakest in macaques (Hecht et al. 2012). Control anatomical tractography in the three species has been performed in geniculostriate and corticospinal tract and no significant differences between the tracts in the three different species have been found (Hecht et al. 2012).

We endorse that hand MNs could be a primate homology of the cerebral connectivity (fronto – parietal circuit) and core mechanism (i.e. the matching execution-observation), with a differentiation of functions (i.e. action recognition, imitation). In particular, primates may have inherited specific sensorimotor structures (i.e. premotor and parietal regions) in specific areas of the brain from a common ancestor (Kaas 2008; Preuss and Goldman Rakic 1991a; Preuss and Goldman Rakic, 1991b). The connectivity between these areas produces the specialization of visuomotor neurons (involved in the visual coordination of arms in space) in MNs. This type of connectivity is probably common to all the primate classes, as it is present both in Strepsirrhines and in anthropoid primates (Preuss and Goldman-Rakic, 1991a; Preuss and Goldman Rakic, 1991b), and in some species, like human being, chimpanzee, macaque and marmoset, has been exploited to code also others' hand movements.

However, given the plasticity shown by MNs, and their high functional value, focusing only on the *classical homology* of this trait may be misleading. The common heritage of primate hand MNs is in fact not centered on specialized neurons activated during perception and execution of grasping actions (Rizzolatti and Matelli, 2003); rather it is based on more generalizable and learnable matching properties of sensorimotor processes regarding the execution and the observation of the interactions between the subject, the hands and the objects (Bonaiuto and Arbib, 2010; Toni et al. 2008). In other words, the primate frontoparietal circuit might transform social visual information into a motor format by virtue of evolutionarily conserved sensorimotor mechanisms tied to the contextual use of upper appendages in a given environment. Hand MNs for grasping actions recorded during laboratory experiments are the adult end-result of a developmental history in which the non-specific motor resonance of the individuals at birth is sculpted and tuned through maturational and learning processes. Therefore frontoparietal circuits of primates might be evolutionary ready to play a role in the *guidance and perception* of visually coding hand movements according to learned rules, where grasping actions are fundamental behavioural acquisitions, needed for the survival of individuals in their natural environments.

Hand MNs in humans, chimpanzees and macaques

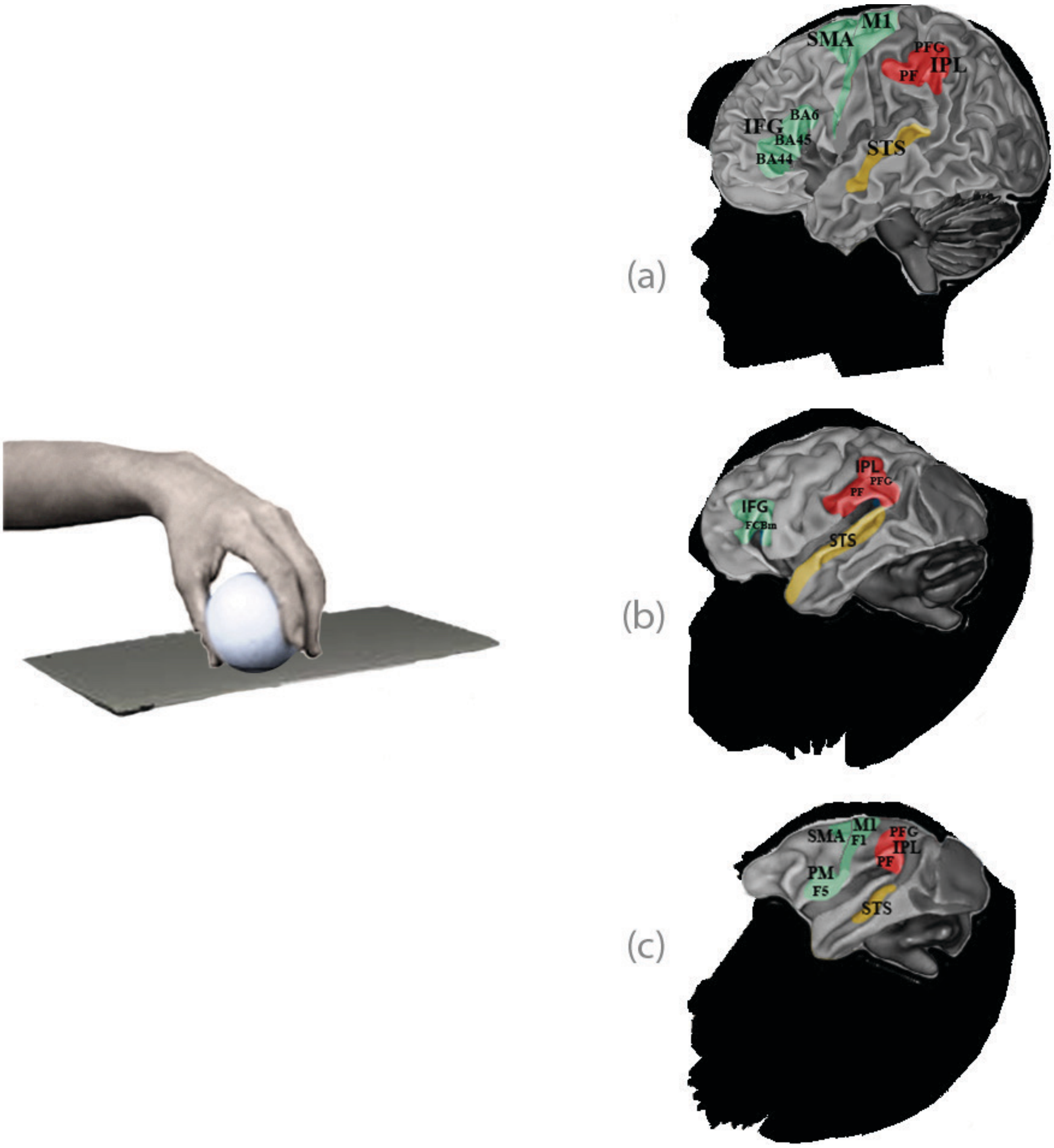


Fig. 1 Specific anatomical regions of human, chimpanzee and macaque brains activate during transitive grasping observation and overlap with regions that activate during the execution of the same grasping actions. We highlight (pre)motor, parietal and sensory areas respectively in *green*. *Red* and y*ellow*. In humans (a), the premotor cortex (PM) and inferior frontal gyrus (IFG) roughly contain Broadman areas BA44 (known as Broca's area), BA45 and BA6, while the rostral part of the Inferior Parietal Lobule (IPL) is thought to correspond to cytoarchitectonic areas PF and PFG. In parietal area, mirror activity has also been found in the anterior sector of Intraparietal Sulcus (AIP). In chimpanzees (b), the IFG occupies the anatomical region FCBm, following Bailey et al. 1950's nomenclature; while in the IPL, mirror responses occupy areas PF and PFD. In macaques (c), PM contain area F5, where mostly hand MNs are located, and in IPL MNs have been found in PF, PFG and in the region AIP of the Intraparietal Sulcus. In humans and macaques, hand mirror responses have also been found in the motor cortex (M1) and Supplementary Motor Area (SMA). The superior Temporal Sulcus (*green*) is a sensorial region which lacks motor properties but is considered part of the hand MNS, because it is thought to be the main source of visual input.

Computational models plus behavioral and neurobiological evidences (Keyser & Perrett, 2004; Bonaiuto and Arbib, 2008; Cook et al. 2014) suggest that hand MNS follow similar developmental trajectory, i.e. similar processes of brain interactions during ontogeny in monkey and human. These models predict that hand MNS emerge throughout motor development as the newborns learn to extract relevant features from manual actions visually perceived, for controlling the hand in the space. At the mechanistic level, MNs are thought to emerge through probabilistic connections and interactions between (pre)motor, parietal and sensorial neurons coding for different aspects of the same actions (Bonaiuto and Arbib, 2008)

These hypotheses are supported from a behavioral standpoint. Evidences show in fact that skilled manual abilities require a long period of maturation (from 1 to 2 years in humans; several months in macaques) and many level of sensorimotor integration. The development and refinement of a successful control strategy for visually guided reaching movements must be accompanied by the execution of appropriate exploratory behavior that involve concurrent and coordinated motor and visual experience. Both monkeys and humans in fact spend considerable time in watching their hand and the early absence of visual feedback during active movements produce sensorimotor rather than purely motor deficits (von Holsten, 2004; Held and Bauer, 1967), suggesting that the emergence of fine perceptual skills related to manual actions are tightly related to the development of associated motor abilities.

It has been hypothesized that these processes of brain interactions related to specific and repeated behavioral experiences epigenetically modify gene expression in MNs during development (Ferrari et al. 2013), suggesting that the physiological role of MNs can be reflected at average at the epigenetic – nuclear level in specific brain regions. This is in line with evidences and models stating that epigenetic regulation associated to developmental plasticity reflect adaptive functional interactions of the brain with the environment during ontogeny (Riedl, 1977; Striedter, 1998; Fishell and Heintz, 2013; Bronfman et al. 2014; Lokk et al. 2014).

Accordingly, we propose that hand visuomotor MNs can be considered *developmental or epigenetic homologues* among the different primate species (Wagner 1989; Rieppel 1994; Striedter 1998). Structures from two individuals or two species are developmentally or epigenetic homologous if they share a set of developmental constraints, caused by locally acting self-regulatory mechanisms of organ differentiation (Wagner 1989). In the case of MNs, the similar primate connectivity of premotor and parietal sensorimotor region suggest that hand MNs may rely on similar developmental brain mechanisms emerging in each individual (Keyser and Perrett 2004; Del Giudice et al. 2009; Iacoboni, 2009; Ferrari et al. 2013; Catmur et al. 2007). In particular, *developmental homology* may rely on common epigenetic mechanisms and similar processes of maturation that produce stable phenotypic results during development (Wagner 1989).

Mouth MNs: evolutionary counterparts across primate species

With the expression *mouth visuomotor mirror neurons* (mouth MNs) we refer to populations of neurons activated by both the visual observation of others' mouth actions, as well as by the performance (execution) of mouth actions oneself. In macaques, and probably other primate species, such as apes and humans, there appear to be two types of mouth MNs: the ingestive and the communicative. MNs may discharge during the observation and the execution of actions performed during an interaction involving the mouth and an object, such as grasping and holding (with mouth), sucking, chewing and breaking (*ingestive* MNs; Ferrari *et al.* 2003). In contrast, *communicative* MNs discharge in response to non-goal-directed intransitive actions, such as lipsmaking or tongue protusion facial gestures (Ferrari *et al.* 2003), facial gestures that are used for communication.

Importantly, *communicative* MNs also fire during the execution of ingestive actions (such as sucking) and interestingly, *ingestive* MNs also fire during communicative behaviour such as lipsmaking and tongue protusion, but with a weaker discharge. Little is known about *communicative* MNs firing during the execution of intransitive communicative actions (Ferrari et al. 2003). The lack of a strict congruence between perception and action in communicative MNs is due partly to the challenge to evoke communicative mouth actions in monkeys during recording sessions. Nonetheless, ethological findings suggest that communicative MNs may be an evolutionary secondary re-functionalization of ingestive MNs, since they seem to share the same neural substrate (Ferrari et al. 2003).

In fact, monkey communicative gestures may be ritualizations of ingestive actions and may derive from the mandibular opening-closing cyclic movements present during chewing and suckling the nipples (MacNeilage 1998; Van Hoof 1962). It has been further proposed that in human infants, early forms of vocal communication, such as babbling, derived from the cyclic open-close mandibular alternation originally evolved from food consumption (MacNeileage 1998: Morrill et al. 2012).

Mouth MNs are very likely to be present also in others primates, such as humans (Buccino et al. 2001; Leslie et al. 2004; Iacoboni et al. 2004), but they have never been, as yet, investigated in chimpanzees. During the observation of facial gestures or during the execution/imitation of the same facial gesture, mirror activation was found in motor (area 6), supplemental motor area (SMA), premotor (BA 44 and BA 45) and parietal (IGF) cortex

(Buccino et al. 2001; Carr et al. 2003). Consistent with these results, Mukamel et al. (2010) recorded neurons with mirror-like properties responding to the execution and perception of mouth actions in not classical mirror areas, such as SMA. Thus, the overlap between areas with specific mouth motor activity and areas responding to similar mouth actions performed by others suggests that, in humans, a number of frontal (premotor and motor) and parietal areas controlling mouth movements may have mirror properties.

In the light of similarities of mouth and hand MNS at the physiological and structural level, the same kind of evolutionary relationship hypothesized for hand visuomotor MNs can be assumed also for mouth visuomotor MNs, with same conserved functions, including later re-functionalizations (i.e. exaptation). In fact, a common feature of mouth MNs across primate species is their pattern of activation in the frontoparietal circuit, where the upper part of temporal cortex is connected to the caudal portion of the parietal cortex, which in turn is linked to the dorsal region of the premotor and possibly motor cortices (Gerbella et al. 2011; Rizzolatti et al. 2014). This would justify the assessment of *classical homology* between macaque and human mouth visuomotor MNs, because these neurons are found in corresponding locations and embedded in very similar pattern of connectivity in both species. Moreover, given that a frontoparietal circuit controlling orofacial movements has been identified even in marmosets (Yao et al. 2002) and prosimians (Kaas 2008), the attribution of *classical homology* highlights that neurons in frontoparietal circuit controlling orofacial movements must have existed in an ancestor of macaques and humans and that they have been recruited during the perception of others' mouth actions under appropriate conditions.

The phenomenon of neonatal imitation makes the existence of mirror responses associated to face movements even more intriguing from an evolutionary point of view. In monkeys, apes and humans, in fact, just hours after birth, newborns are capable of imitative abilities when the experimenter or the caregiver performs a mouth gesture, such as lipsmaking or a tongue protusion (Meltzoff and Moore, 1977; Ferrari et al. 2003; Myowa-Yamakoshi et al. 2004; Ferrari et al. 2006; Bard et al. 2007). Moreover, at 36 hours from birth, human newborns discriminate and reproduce three different emotional facial gestures (happiness, sadness and surprise) expressed by a model (Field et al. 1982; see also Simpson et al. 2014).

Given that face-to-face interactions typically involve effectors (such as the mouth and tongue) that neonates cannot visually access, processes of sensorimotor mapping between observation and execution of mouth gestures is crucial in neonatal imitation (Meltzoff and Moore 1977; Meltzoff and Borton 1979), suggesting that some rudimentary observation/execution matching system may be present at birth or shortly after birth in human, ape and monkey brain (Ferrari et al. 2012; Vanderwert et al. 2015).

As confirmation of this hypothesis, electroencephalogram (EEG) studies in macaque newborns have found, similar to human infants, neural activity reflective of brain mirroring (Ferrari et al. 2012; Vanderwert et al. 2015). Specific variations in alpha frequency in EEG studies have been recorded in the context of neonatal imitation (Ferrari et al., 2012). In particular, it has been observed in newborn monkeys that during the observation and imitation of facial gestures specific frequency bands of the EEG tend to reduce in amplitude. Such phenomenon is recorded over the central-frontal electrodes and has been termed *mu rhythm* (Ferrari et al., 2012). The mu rhythm has been known for long but in the last decades has been investigated more in depth at it seems to represent an indirect marker of the MN system (Marshall and Meltzoff, 2011). The finding of a mu rhythm in newborn monkeys and its sensitivity to early social experience (Vanderwert et al. 2015) provides important information about the presence of a mirror mechanism at birth as correlated to infant synchronous dyadic communication.

As *mu rhythm* signals are considered to be associated with the activation of sensorimotor neural areas (Vanderwert et al. 2013), a plausible interpretation is that a rudimentary visual-mouth mirror mechanism is present from birth, allowing the matching between the observed mouth actions and the associated facial expressions with the display of the same behaviours (Nagy and Molnar, 2004; Bonini and Ferrari, 2010; Tramacere et al. in press). The presence of *mu rhythm* suppression during mouth gestures observation by macaque and human neonates, is even more informative if we consider that a similar elettrophysiological effect is not associated to hand actions perception in the same perinatal period (Ferrari et al. in preparation). If confirmed, this result will bring support to the idea that the neural networks involved in facial perception has peculiar properties of developmental 'readiness'.

Interestingly, a kind of visual orientation is possible even in occipital cortical blind infants, suggesting that early visual abilities might rely on subcortical structures as well (Dubowitz et al. 1986). A neural circuit involving both neocortex and subcortex may thus participate and contribute to early imitative responses in monkeys, ape and humans.

The matching between face perception and execution may have been favoured during primate evolution, in the light of their involvement in parent – infant affiliation and attachment (Simpson et al. 2014; Tramacere & Ferrari, in press). Our hypothesis entails that MNS (together with specific subcortical pathway) is actively involved in and mediate these processes.

Audio – vocal MNs: the exaptation of old structures

We next discuss *audio-vocal mirror neurons*, populations of neurons activated by others' vocal sounds and likewise activated by vocalization production. These neurons have been directly investigated in songbirds and indirectly inferred in human beings (Prather et al. 2008; Pulvermueller and Fadiga, 2010)

In avian species, auditory responses of single identified neurons were investigated in swamp sparrows and Bengalese finches. In song sparrows, neurons in HVC projecting Area X (HVC $_{(X)}$) fired during song playback (Prather et al. 2008) *[See fig. 2].* These neurons appear to exhibit a highly selective firing pattern, responsive only to one song within the bird's repertoire, namely the primary song type, intended as the first singing-auditory activity found in a HVC$_{(x)}$ cell for one of the bird's own songs (Prather et al. 2008). Unlike these neurons, the HVC$_{(RA)}$ neurons, projecting to the robust nucleus of the arcopallium (RA), were entirely unresponsive to stimulation during the listening condition (Prather et al. 2008). Thus, it seems that avian audio–vocal MNs, being present only in HVC neurons projecting to Area X, are part of the so–called Anterior Forebrain Pathways (AFP), connecting HVC, Area X (a basal ganglia nucleus), DLM (a thalamic nucleus) and LMAN. Several lesion experiments clearly show that the AFP, considered to be analogous to the cortico–basal ganglia-thalamic loop of mammals (Graybiel, 2005; Jarvis et al. 2005), is necessary for song learning in juvenile birds (Bottjer et al. 1984).

Earlier studies on mirror X–projecting neurons proposed that they might serve the function of corollary discharge of motor commands and have a specific role in communication (Prather et al. 2008; Keller and Hahnloser, 2009). Corollary discharge is thought to play a role in the distinction between self-made and others' signals: sensory receptors are, in principle, indifferent to the nature of the input, not distinguishing whether they come from own movements or from perceived others' action (Crapse and Sommer, 2008). As a consequence, animals would face an ambiguity in encoding the environment that could have disastrous effects. Therefore, corollary discharge can contribute to the construction of a reliable perception of the surrounding social world. However, additional experiments are necessary to confirm this hypothesis and understand whether audio-vocal MNs are simply the result of auditory feedback or whether they have a role in guiding sensorimotor learning.

There is now evidence, obtained through fMRI and TMS techniques, showing that mirror mechanisms are present also in human beings during vocal/speech perception (Pulvermüller and Fadiga 2010). Spoken words have been shown to activate neural populations in the superior temporal gyrus (traditionally known as Wernicke's area) and subsequently in part of the inferior frontal gyrus (Ba44, also called Broca's area) (Pulvermüller et al. 2003). Wilson et al. (2004) and Pulvermüller et al. (2006) showed, with TMS and fMRI respectively, that passive perception of syllables strongly led to increased activation in Broca's area, converging on the observation that activation was greater for speech than non-speech sounds.

Consistent with the view that speech perception involves the motor system in a process of auditory–to–articulatory mapping to access phonation with motor properties are the findings obtained through TMS techniques: listening to speech increases motor evoked potentials in the lip muscles, while listening to non–speech sounds does not (Watkins et al. 2003; Watkins and Paus 2004). Similar perceptual enhancements of motor excitability have been obtained also in the tongue area of the motor cortex and tongue muscles (Fadiga et al. 2002; Fadiga et al. 2005). On the basis of these observations some authors (Hauk et al. 2008; Schomers et al. 2014) attempted to establish the cognitive advantages of a perceptual processing that overlaps with neural circuits devoted to motor execution, rather than having different channels for perceptual input and motor output. Through TMS pulses upon the representation of lip and tongue muscles in motor cortex, the efficiency in word–to–picture recognition task were measured. The results suggested a causal role of motor cortex in word auditory perceptual recognition (Schomers et al. 2014).

Given that audio–vocal MNs have been investigated in humans and songbirds, the assessment of homology between them should be traced back to their distant ancestors. Sauropsides (reptiles and birds) and synapsides (mammals) belong to the amniotes group, which split from each other in the Carboniferous about 310 mln of years ago (Coates et al. 2008). During this period—after the split, until today—these groups (reptiles/birds and mammals) have evolved independently. Thus, in order to investigate a possible homology between avian and human audio – vocal MNs, it should be established whether these types of sensorimotor neurons are present in corresponding locations of the brain of animals outside of amniotes, such as amphibian.

Unfortunately, we lack information on amphibian based on single-cell recording. Furthermore, avian MNs have been found only in songbirds (Prather et al. 2008), and are very likely absent in mostly suboscines (no-singing species).

Suboscines in fact are known for their innate calls, associated to the midbrain/brainstem centres, and their lack of both encoding and production of vocalization acquired from conspecific (Nottebohm 1972).

Actual and potential vocal learners and audio – vocal MNs

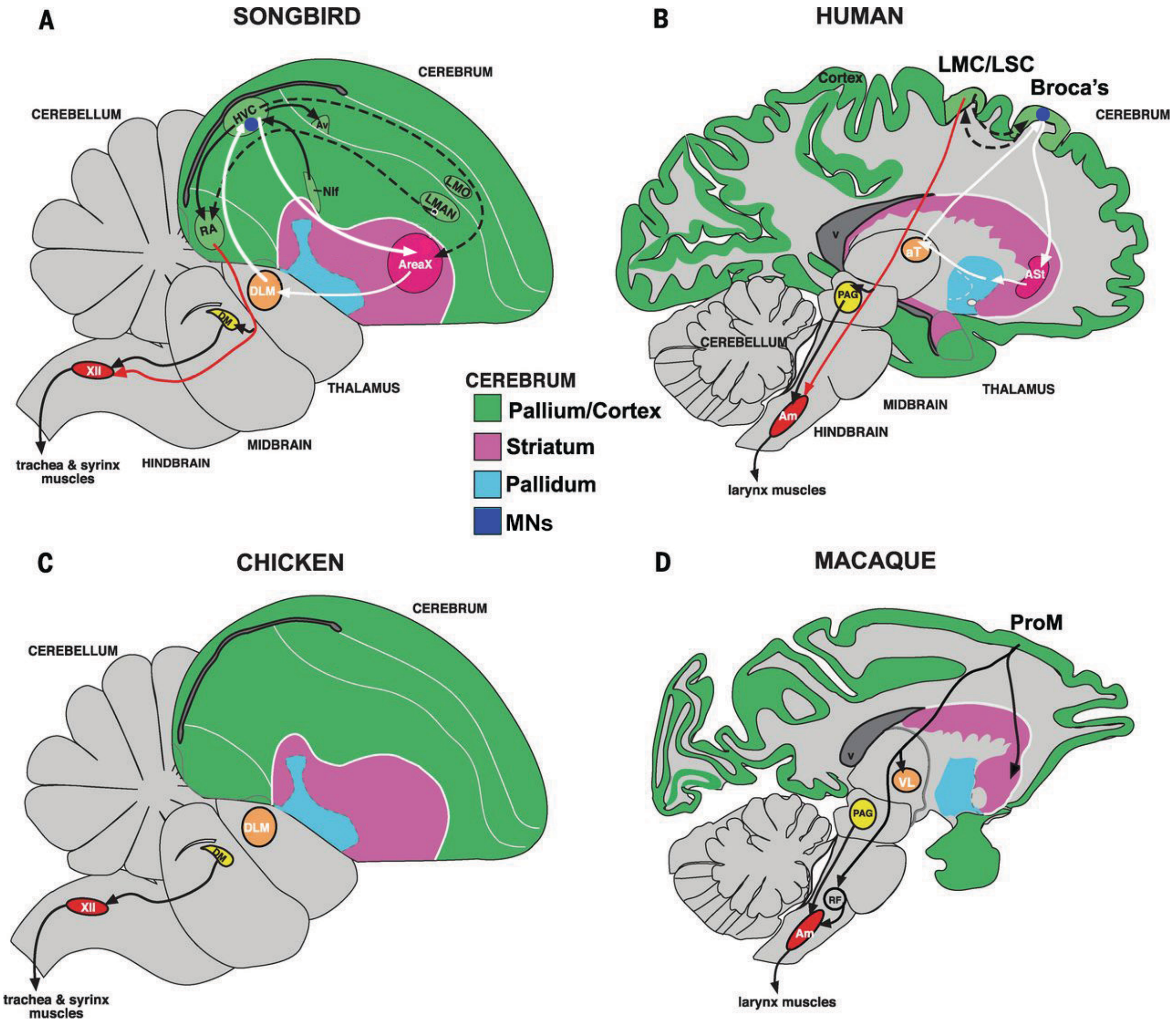


Fig. 2 Picture modified by Pfenning et al. 2014. Songbirds and humans, both vocal learners, have a similar pattern in the way premotor/motor areas are connected with brainstem in the establishment of learned vocalizations. In songbirds (a) the motor nucleus RA connects with motoneurons of the syrinx for the production of song, while a preparatory and modulatory role is played by the premotor nucleus HVC. Similarly, in human (b), the face area of the primary motor cortex in BA 4 (the region involved in laryngeal motor control) connects to the Nucleus Ambiguous (Amb), while the Broca's area, a premotor region, is thought to play a more anticipatory and planning role. Audio-vocal MNs have been found in HVC and Broca's area, respectively in songbirds and human.
In non-singing birds or in songbirds' female,c), which lack vocal learning, the circuit controlling innate calls involves connections between Periacqueductal Grey (PAG) and motoneurons of the syrinx and larynx. A similar circuit has been described in non-human primates, such as macaque (d), where the connections between PAG and the motoneurons of Amb are necessary, together with the Anterior Cingulate Cortex (ACC), to produce emotional spontaneous vocalizations. In contrast, the lateral part of the cerebral cortex of nonhuman primates, the premotor cortex, has a minor role in voluntary control, despite that, in its most lateral part, it has motor representations of the mouth and larynx. Nevertheless, both female songbirds and macaques can be trained to voluntarily vocalize and consequently to establish the connection between the premotor cortex and motoneurons, and therefore have the capacity to develop audio-vocal MNs in the premotor regions.

Suboscines also lack the forebrain cell clusters where audio – vocal MNs are located and that, integrating sensorimotor signals, control song learning in oscines (song birds) (Nottebohm, 1972). Given the impossibility of a structural homology of MNs (or the neural circuits where they are embedded) across the very different species of synapsides and sauropsides, the assessment of homology between birds and primates audio – vocal MNs seems unsupported.

As an another possible explanation, *convergent evolution* of avian and human audio – vocal MNs. Convergent evolution is the process whereby organisms not closely related independently evolve structurally similar traits as a result of having to adapt to similar ecological niches (Stern 2013). It is a functional analogy between even complex traits, not due to any structure shared with a common ancestor (like echolocation in bats and some birds).

Convergent traits should be understood as modifications of primitive conditions (Shubin et al. 2009). In this respect, it is worth of notice that in humans, audio–vocal mirror responses have been localized in the Broca's area (BA 44) a premotor region of human frontal cortex (Pulvermüller et al. 2006; Wilson et al. 2004). In songbirds, MNs have been localized in HVC (High Vocal Center), a nucleus with premotor properties of the pallium (Kozhevnikov and Fee, 2007). This sector of the avian pallium, as the corresponding frontal sector of mammal neocortex, receives visual and auditory signals from the thalamus (Jarvis et al. 2005). It also processes the same types of sensory information as the mammalian neocortex and gives rise to important descending projections to the motoneurons of the brainstem and spinal cord, involved in the voluntary control of vocal tract and respiration. Finally, like the mammalian neocortex, the pallium serves a crucial role in song sensorimotor learning (Jarvis et al. 2005) *[See fig. 2]*.

Some scholars have also highlighted the functional similarities between the neurophysiological properties of avian HVC and human Broca's area (Merker and Okanoya, 2007; Doupe and Kuhl, 1999), both are implicated in coding mouth and syringeal movements, and in high-order decoding of respectively syllable and speech production.

From an evolutionary point of view, songbirds and mammals could have co-opted a similar primitive neural structure with corresponding functional characteristics for the emergence of vocal learning (Bolhuis et al. 2010). According to this hypothesis, the development of audio-vocal MNs both in humans and songbirds could be understood as a neural reuse (Anderson, 2010) or an exaptation (Gould and Vrba, 1982; Pievani and Serrelli 2011) of the primitive properties of avian and human premotor neurons: once established the connections between premotor and motor neurons of the brainstem for vocal execution, Broca's and HVC neurons may have generalized their firing response to the perception of conspecific vocal sounds.

Moreover, based on the hypothesis that similar avian and mammalian brain areas may express similar gene sets (Jarvis et al. 2013; Pfenning et al. 2014), it is possible that Broca and HVC's tissues similarity is due to a common molecular pathway. It has recently been revealed by molecular studies that one or more genes underlying a complex trait could underlie convergent evolution across species, even species separated by hundreds of millions of years from a common ancestor (Pfenning et al. 2014). Examples of this phenomenon are the echolocation in bats and cetaceans (Liu et al. 2010), the emergence of electric organs in different lineages of fishes (Zakon et al. 2006), and skin colours in different mammalian species (Arendt and Reznick 2008).

In relation to vocal learning, convergent changes in amino acid sequences in avian species and in *Homo sapiens* have recently been reported (Wang 2011; Zhang et al. 2014). In addition, preliminary molecular studies performed on micro-dissected song control nuclei and human post mortem samples report a remarkable convergent genetic expression between avian RA and human laryngeal motor cortex and between Area X (a striatal nucleus) and putamen (Pfenning et al. 2014). A fascinating hypothesis is that a common genetic regulation could be at the basis of the premotor specialization of, respectively, the pallium in songbirds and the cortex in *Homo sapiens*, and that it is also involved in the general emergence of neuronal activities with mirror responses. This would justify the assignment of the hypothesis of *factorial homolog* for avian and human audio–vocal MNs; factorial homology stands for two or more traits that are not historical homolog (because they cannot be trace back to their common ancestor, having evolved independently from different ancestral structures), rather are developmental homolog, having been independently co-opted the same developmental module, i.e. the same generative gene network module (Minelli and Fusco 2013). Summing up, novel complex adaptations may have emerged in the natural history of animals by re-using or functional extending of old homologous anatomical and genetic structures, which were deeply shared to different degrees (Wray and Abouheif 1998; Shubin et al. 2009), and that are also implicated in the development of the matching between vocal execution and auditory feedback. Future studies may shed light on this question.

The Mosaic Evolution Hypothesis for mirror neurons

The data we reviewed suggest that the mirror system is not a single evolutionary trait. Rather, we propose that the mirror system, while sharing a core action-perception matching mechanism, is actually a set of interrelated traits, each with an independent evolutionary history reflecting unique selective pressures, much like human language and the mammalian isocortex (Fitch 2012; Barton and Harvey, 2000). MNs evolution may then be interpreted as a case of “mosaic evolution”. Mosaic evolution refers to evolutionary changes that occur in some body parts or systems without simultaneous changes in other parts. In other words, complex traits may evolve at varying rates within and among species (Carroll 1997; Minelli and Fusco 2013). Some traits may be very old (i.e., existed for numerous generations) and may be changing slowly through gradual evolution, while other parts could be recent (i.e., appearing in only recent generations) and may be changing more quickly. Some traits may exhibit ancient and homolog structural constraints between the considered species; other parts may exhibit more recent functional changes. With mosaic evolution, a major morphological and behavioural transition of a trait (for instance, bipedalism in the sub-family of hominins) can occur in the phylogenetic tree through the exploration of several adaptive solutions, each one being a different combination of primitive and new sub-traits.

Here we hypothesize that in the case of MNs, the dynamic of their phylogenetic emergence in the different species suggests that during evolution different types of action have been selected – perception neural mechanisms – producing various MNs developmental dynamics, slightly differential physiological properties and patterns of connections with other neuronal systems for each of them. Indeed, MNs seem to develop with different timing and dynamics in relation to the effector hand, mouth or vocal tract, and in relation to the species – specific environmental demands.

We will further elaborate this hypothesis in the next sections.

Multiple re-uses in manual gestures evolution

The evolutionary history of hand visuomotor MNs appear to be the result of a series of events in which a combination of uses, reuses, exaptations and further specializations might have occurred. The existence of neurons responding to the observation and execution of hand actions is associated with the evolution of the forelimbs, which in turn is related to the neural control of muscles and bones evolved for locomotion.

In arboreal primates, who rapidly throw themselves from one branch to another, locomotion involves also particularly developed reaching and grasping ability. Arboreal primates have thus evolved neural circuits for highly mobile forelimbs, which allow these animals to flex their arms and, through a highly specialized hand (and, for some, tails), to grasp in many planes (Schmitt 1998).

In evolution, it is quite common for neural circuits conserved in relation to one function to be exploited or reused for another function, often without losing its original function (Anderson 2010). Circuits can continue to acquire new uses after an initial function is established and the acquisition of new uses needs not to involve unusual circumstances (such as loss of established function) (Anderson 2010; Pievani and Serrelli 2011). Hence, while there has been a progressive increase in forelimbs system complexity during primate evolution and there are many differences between primates and other mammals' locomotion (Schmitt 1998), many basic neural features associated to limbs control have been preserved across vertebrates (Falgairolle et al. 2006). The motricity of the limbs is under the control of supra-spinal centres and of specific propriospinal systems (Lundberg 1999). This neuronal network has been identified during both foot locomotion and prehension tasks in different mammals, such as cats, monkeys and humans (Vilensky 1987).

However, a widely accepted view suggests that primates evolved fine skilled hand actions through the potentiation of a direct pathway between premotor and motor cortex and motoneurons of the spinal tract controlling the limbs (Yuste *et al.* 2005; Lemon, 2008). Primates thus achieved a sort of "cortical dominance", with cortical circuits controlling spinal activity that allow efficient forelimbs movements (Duysens and Van de Crommert 1998; Yuste et al. 2005; Lemon 2008).

This derived and specialized trait can be dated back to around 60 million years ago, when the evolution of Strepsirrhines took place: lemurs, galagos and tarsiers, in fact, can use their hands for locomotion, but also for foraging food and other prehensile activities (Fragaszy, 1998). Neuroanatomical studies on prosimians confirmed that those species have a frontoparietal circuit, specialized for the coordination of the hand in the space and connected with the spinal tract (Preuss and Goldman – Rakic 1991b; Lemon 2008; Kaas 2008).

Species with more complex social dynamics, such as diurnal species (i.e. *Lemur catta*), might acquire mirror responses in cortical regions specialized for hand movements. Even though the social cohesion of Strepsirrhines in natural environments remains limited (with few exceptions) compared with that of anthropoid primates (Goodman et al. 1993), their life is far from being socially isolated. Although many lemurs and galagos species are classified as "solitary foragers" (Bearder 1999), they still exhibit some level of social organization: some of them live in pairs (Thalmann 2001), others show forms of gregariousness (Gursky 1995; Warren and Crompton, 1997), and others may even live in multimale/multifemale groups (Warren and Crompton 1997). It is possible that the enriched social life of some prosimian species might have favoured the emergence of cortical circuits specifically devoted to the decoding visual information regarding others' behaviour. Under these circumstances, parietal-premotor circuits responding to action observation could have emerged as a consequence of specific social pressures.

However, it is reasonable that with the evolution of primate societies, as observed in New

World monkeys (Lazaro-Perea 2001), hand visuomotor MNs emerged constantly as neurons related to relevant social cognitive functions. Since MNs development requires a coupling between neural circuits for the execution and the observation of the same actions, they may have been acquired during their highly social activities, which required proximity, close social interactions and coordination, such as during grooming sessions, social foraging, defensive behaviours, and cooperative breeding (Lazaro-Perea 2001). Similarly to the pioneering study in marmosets which described the presence of hand MNs recorded through single – cell recording (Suzuki et al. 2014), we expect that neurons with similar mirror properties would be present also in other new-world monkey species such as tamarins, squirrel monkeys and capuchin monkeys. Since hand visuomotor MNs have been found in four species of macaques and one of chimpanzees, we expect that their existence will be verified and confirmed also in other species of Old World monkeys (macaques, mandrillus, etc.), and in apes (chimpanzees, gibbons, gorillas, orangutans).

MNS, as most neural structures, show a high degree of plasticity. One interesting example is the use of tools that can modify their tuning, expanding their response to other types of actions. This finding may have important implications from an evolutionary point of view. It has been discovered that tool use recruits similar neural areas involved in execution and perception of hand actions in humans (Järveläinen et al. 2004; Rochat et al. 2010), very likely in chimpanzees (Hecht et al. 2012; Hecht et al. 2013) and in macaques (Ferrari et al. 2005; Umiltà et al. 2007). In macaque, where single-cell recording is experimentally feasible, neurons in premotor cortex responding to the observation of a particular tool use have been found to be specific for this task (Ferrari et al. 2005; Rochat et al. 2010). This response is stronger than that obtained when the monkey observed a similar action performed with a biological effector, such as the hand (Ferrari et al. 2005). These findings suggest that tool-use neurons could be the result of a plastic co-option and functional shift of pre-existing hand-grasping or hand-reaching MNs.

Consistent with this interpretation are the experiments with macaque tool use. Although these animals do not typically use tools in natural environments, after two weeks of extensive training they are proficient at obtaining food with a rake (Ishibashi et al. 2000). Interestingly, during and soon after this training, neuroplastic change were observed in the monkey parietal region, where hand MNs are located, such as macroscopic expansion of grey matter, axogenesis and synaptogenesis (Hihara et al. 2006), and these changes were accompanied with elevated expression of immediate early genes (Ishibashi et al. 1999), and of neurotrophic factors (Ishibashi et al. 2002a; Ishibashi et al. 2002b).

These findings suggest that once monkeys face a novel cognitive challenge such as recruiting or handling sources of food with tools, hand MNs together with canonical neurons (related to objects observation and manipulation – Rizzolatti and Fadiga, 1998) and bimodal neurons (related to both visual and tactile inputs processing – Iriki et al. 2001) in premotor and parietal cortices (Grove and Coward, 2008) undergo structural and molecular changes. Whether these plastic changes are responsible for the emergent specificity of tool-responding MNs investigated in macaque monkeys during laboratories experiments requires careful future work.

These experiments on macaque tool use also suggest that monkeys possess latent cognitive abilities that can be realized by exposure to the proper enabling environment. Further investigations are needed to understand whether the recently observed populations of tool – user macaques in their natural environment (Gumert and Malaivijitnond 2013) are experiencing the same types of neural and molecular changes observed during lab experiments.

The evidence that shows the role of plasticity in major evolutionary changes (Standen et al. 2014) in general in the deployment of the MN system for tool use in particular, suggests that early hominids may have experienced similar neural and molecular changes and re-organizations during their phylogeny, as tools are thought to be an integral part of the hominid environmental niche (Matsuzawa 2014; Iriki and Taoka 2012). In human evolutionary history, tool use could have given rise to mutual interactions between individuals, groups and their environments (resulting in a process of social and ecological niche construction: Iriki and Taoka 2012), where phenomena of molecular plasticity might pave the way to new modalities of genes expression that in turn underlie the reorganization of neuronal connectivity between mirror areas.

To summarize, hand MNS are an important property of monkeys, apes and humans, inevitably and reliably resulting from the evolution of the regulatory structures of the primate brain and the associated environmental niches. In these cases, the explanation of the origins of MNs' stability across development in terms of natural selection is unwarranted, because the developmental plasticity of the primate brain is sufficient to account for their emergence during ontogeny. However, natural selection has probably contributed to the maintenance of hand MNs (and subsequently of tool-responding MNs) during phylogeny, as they may be associated to relevant biological functions, such as manual gestures recognition and imitation in a social and communicative context.

Natural selection on facial coordination in neonates

In anthropoid primates (i.e. New World monkey, Old World monkey, ape and human beings), visual preference for faces (Leopold and Rhodes, 2010; Burrows 2008) involves a process of selective attention toward specific markers, such as eyes and mouth (Schmidt and Cohn, 2001). The mouth seems to be an important communicative component (Kano et al. 2012). Yawning, for example, is considered a signal for conspecifics (Smith 1999), smiling and lipsmaking are affiliative cues for humans and nonhuman primates, respectively (van Hooff 1962; Ferrari et al. 2003), and the exposure of canines signals a low–grade threat (Hadidian 1980). In primates' natural environments, where faces seem to be salient stimulus for a variety of interactions—including conflict resolution, territory defense, sexual signals, parent-offspring interactions, social integration and communication (Bradbury and Vehrencamp 1998)—it is plausible that face-to-face interactions tuned, through processes of Hebbian learning and synaptic competition, sensorimotor circuits for facial and mouth proprioception and motor coordination on the one hand, and specialized circuits for perceiving and interpreting others' facial signals, on the other hand (Schmidt and Cohn 2001).

Since most of the parietal-frontal circuits controlling hand and mouth movements have a similar frontoparietal pattern of connections in Old-World monkeys and prosimians, it is possible that also in prosimians some forms of communications based on facial displays might rely on these circuits that operate through neural mirroring in similar ways to macaques, chimpanzees and humans. Although still speculative, it is possible that dyadic communicative episodes, such as the relaxed open mouth display used as a play signal in ring-tailed lemurs (Palagi et al. 2014), may have led individuals of those species to activate frontoparietal circuits for facial and mouth movements (Preuss and Goldman – Rakic, 1991b; Stepniewska et al. 2005) during face perception, producing the first forms of mirror-like action-perception coupling in the primate brain. Populations of neurons responding both to the execution and observation of specific mouth actions may thus have evolved in individuals of some social and diurnal Strepsirrhine species, in contrast to nocturnal species that rely more on olfactory cues, and who possess primary motor and premotor areas very similar to those of anthropoid primates (Kaas 2008; Preuss and Goldman – Rakic 1991a; Preuss and Goldman Rakic 1991b).

As a matter of prediction, we speculate that mechanisms of perceptual-motor coupling related to face and mouth processing might have emerged already by about 60 million years ago in some early primates, such as in prosimians or Strepsirrhines, which include the Lemuroidea and the Lorisoidea. Some of these species experience a low but significant level of face-to-face interactions that is translated into a proper grouped organization and related intraspecific communication (Klopfer and Boskoff 1979; Kappeler and van Shaik 2002), such as for example playful facial signals (Palagi 2009).

However, in most prosimians complex communicative signals based on face-to-face exchanges have been observed primarily during late adolescence and adulthood, and not during the earlier postnatal periods, where males and females acquire quite stereotyped frequencies of play, grooming sessions and reproductive behaviours (Doyle 1979). Indeed, parents of galagos, lemurs and tarsiers spend very little time with their infants, who mature precociously (Klopfer and Boskoff 1979). Given the nature of prosimian maternal behaviours, with babies carried by mouth or on the mother's back, the number of mother-infant face-to-face interactions appears low and less based on facial gesture exchanges than anthropoid primates (Klopfer and Boskoff 1979). Together these observations (and mainly the degree of neoteny in nonhuman primate species) may have important implications for brain development, and more specifically for how cortical and subcortical networks evolved in order to sustain complex social interactions based on facial gesturing. Therefore, although prosimians may develop mouth MNs during adolescence or adulthood, it seems unlikely that these species could develop mouth visuomotor mirror responses in the first phases of development.

The increasing social demand (i.e. increase group size and social complexity) that has been verified during the transition between ancestral prosimians and the lineage leading to the modern anthropoid primates about 40 million years ago (Schultz and Dunbar 2007; Dunbar 2010) may have favoured individuals more efficient in coordinating their own facial and mouth movements in response to facial gestures of conspecifics, including caregivers, rivals, and companions. Changes in social niches, such as the growth of multilevel societies and more complex dynamics of parental and social bonding (Schultz and Dunbar 2007; Dunbar 2010), may have produced a stronger selective pressure on anthropoid primates' facial recognition and expression capacities underpinned by MNS.

In other words, the increasing neoteny and a progressively more demanding social niche may have produced a new selective pressure on individuals who were more efficient in intraspecific communication (Schultz and Dunbar 2007; Dunbar and Shultz 2007), favouring the coordination of dyadic facial events, such as those occurring during precocious affiliative communicative situations. Consistent with this interpretation, anthropoid primates spent much time in face-to-face interactions in the neonatal and early postnatal developmental periods, and exhibited some of the most intricate facial displays and most complex facial musculature in all mammals (Burrows 2008).

During primate phylogeny, individuals may thus have gained fitness by solving the so-called *correspondence problem* or *perceptual-motor translation problem* (Heyes 2010; Meltzoff and Decety 2003; Brass and Heyes 2005), which refers to the capacity to coherently respond with one's own facial expression to that of another individual, without directly being able to observe one's own face.

The early development of mouth visuomotor mirror neurons may have been consequently *experientially canalized* (del Giudice et al. 2009; Ferrari et al. 2013) during the evolutionary history of primates, leading to a major readiness of neural circuits associated to mouth observation coding in respect to those devoted to hand visual processing. As explained in previous sections, mouth mirror mechanisms seem to be already functional at birth, as they are probably crucially involved in the mechanism of neonatal imitation in monkeys, apes and humans (Meltzoff and Moore, 1997; Myowa-Yamakoshi et al. 2004; Ferrari et al. 2006; Ferrari et al. 2012; Simpson et al. 2014). Although the coupling between perceptual cues and motor activation is not fully developed and refined at birth, the wiring between frontoparietal and temporal sensorimotor regions (Smyser et al. 2011) and the newborn sensitivity to visual facial cues likely provide the neurobiological basis upon which neonates rely to associate their facial motor output with mouth visual input during interactions with the caregiver (Soussignan et al. 2011; Ferrari et al. 2013).

Genetic, developmental and environmental factors might operate in concert in order to progressively achieve a fully functional MNS, that will continue to expand, reorganize and connect through associative processes and cultural demands during the lifespan of the individual (Ferrari et al. 2013). More generally, *experiential canalization* indicates that evolution promoted the course of development to be reliably influenced by specific stimuli, although variations are possible because of particular or unusual events; in other words experiential canalization produces a situation where the output is stable despite subtle changes in input or developmental trajectories (Miller 1989; Gottlieb 1991; Jablonka and Lamb 2002; Dor and Jablonka 2010).

In the phenomenon of canalization, genetic and epigenetic developmental variations (generated by developmental and social niche construction) can contribute to the stabilization of cognitive/behavioral development (Gottlieb 1991). However, although specific molecular factors could be involved in the emergence of mouth visuomotor mirror responses in the neonatal phase (Provencal et al. 2012), the role of caregiver – infant interactions seems to be fundamental in initiating and dynamically regulating MNS activation (Ferrari et al. 2013; Simpson et al. 2014).

To summarize, mirror responses related to face and mouth actions may have already emerged in frontoparietal cortex of adult prosimian species. However, our proposal is that only in anthropoid primates, such as Old World monkeys, apes and subsequently *Homo sapiens*, the increasing social selective pressures, neoteny and the progressively more tighten nature of the mother – infant relationships produced ready mouth mirror responses at birth, expressed already during the neonatal period of development (Tramacere & Ferrari in press).

MNs in the tree of life

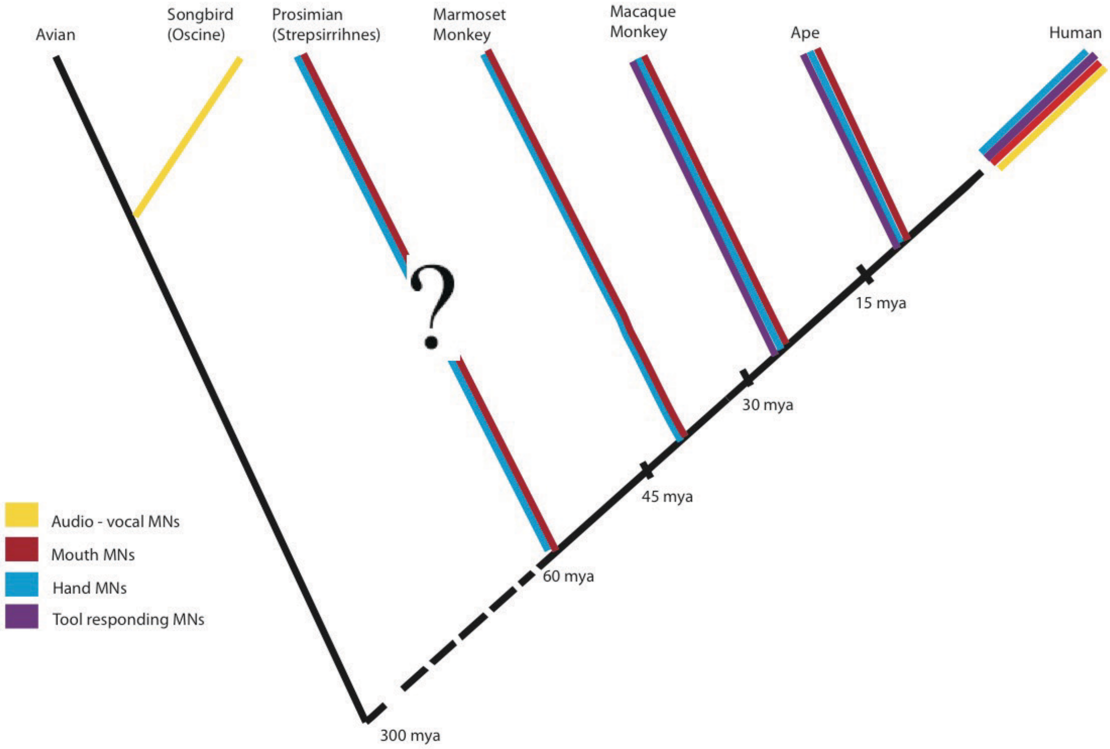


Fig. 3 Mirror neurons can be categorized in relation to the effector with which executed actions are performed (hand, mouth or vocal tract) and the modality of the sensorial input in which the same actions are perceived (vision or hearing).

We show that *hand MNs* (blue lines) are a trait observed in humans, chimpanzees, macaques and marmosets. Further, a violet line indicates the later exaptation of circuits devoted to hand MNs for tool – use and the consequent existence (after a sensorimotor training with tools in the lab – Ferrari et al. 2005) of *tool – responding MNs* in macaques, humans and likely in apes.

Mouth MNs (red lines) have been reported in humans and macaques, and they are likely present also in chimpanzees and marmosets. We further propose that mouth and hand MNs may be present also in prosimians (red and blue lines with questions mark). *Audio – vocal MNs* (yellow line) have been found in human and songbirds.

Developmental plasticity in vocal communication

Audio-vocal MNs seem to be the result of an evolutionary process in which the ancestral amniotes' (reptiles, birds, mammals) sensorimotor circuits controlling innate calls were extended in the establishment of the corticospinal connection for the execution and perception of learned vocalization. In the majority of bird and mammal species, however, vocal behaviour is mostly innate, spontaneous and localized in what (in traditional terms) has been called the "paleoenchephalon" (Nottebohm, 1972; Reiner *et al*. 2004). In contrast, the evolutionary emergence of vocal learning circuits is limited to some species of birds, the songbirds, and to *Homo sapiens* (Nottebohm, 1972; Jarvis *et al.* 2005), and it is supposed to involve the projection of a neural pathway from the premotor and motor cortex to the brainstem *[See fig. 2]*. As explained in section 2.3, it is then in the avian and human premotor regions (HVC and Broca's area) that audio-vocal mirror mechanisms have emerged as neurons related to executive and perceptual vocal coding.

The phenomenon of imitation is central to vocal learning: forming a model of the heard vocal sound is in fact crucial in order to develop song or spoken language (Jarvis 2004). In addition, auditory feedback has an important role, because it is instrumental for the evaluation of the vocal skills achieved by the individuals (Konishi 1965). Thus, it is plausible that the neural matching between conspecifics' auditory input and vocal output responsible for the emergence of these types of MNs coincided with the emergence of the first form of vocal learning in both songbirds and primates.

The distinction between vocal and non-vocal learners is not sharp, due to plasticity. In fact, in several species of vertebrates (e.g., mice, frogs, reptiles, birds) calls are highly stereotypic and associated with emotional drives (Feng et al. 2006; Jarvis 2004; Kikusui et al. 2011). They are under the control of subcortical brain structures, which reveal little to no voluntary control (Nottebohm 1972). While monkeys seem to have a similar type of control (Jürgens, 2002), more recent studies reveal that monkeys' vocalizations and the capacity for voluntary control are more nuanced (Fogassi et al. 2013; Coudè et al. 2011; Hage et al. 2013). Experimental evidence suggests that monkeys can be conditioned to modify and control their vocalizations with training (Coudè et al. 2011; Hage et al. 2013). Macaque monkeys can achieve a significant level of vocal control and learn to voluntarily emit a vocalization following a vocal operant conditioning training (Coudé et al. 2011). After such training, these authors found neurons activated specifically during the production of voluntary vocalizations.

In this respect, it is worth noting that many songbird species show a strong sexual dimorphism, with sexual selection and female choice, so that only males learn to vocalize and develop the proper song nuclei necessary for the emergence of songs (Nottebohm 1972).

Nevertheless, if females are treated with hormones such as estrogens and testosterone, they can be conditioned to undergo the sensorimotor phase responsible for learning complex songs (Nottebohm 1980).

These observations suggest that vocal learning is not an *on-off* mechanism of development that emerges rapidly in some species, but that it requires developmental construction. Studies in birds have demonstrated that the definition of a vocal or non-vocal learner species is not clear-cut; rather there are latent cognitive abilities related to learned vocalization that can be triggered by the proper cues (learning conditions) and/or physiological influences (hormonal regulation). In other words, vocal learning seems to be a plastic behaviour, because the effect of specific exogenous or endogenous environmental conditions can activate specific circuits associated with it.

Conclusions

MNs are a discrete population of neurons responding to execution and perception of the same actions and basically forming a network of interconnected areas in the frontoparietal regions of the brain. We considered different categories of MNs according to the effector performing and sensory channel perceiving the mirrored actions, thus obtaining three subgroups of MNs: hand visuomotor MNs, mouth visuomotor MNs, and audio-vocal MNs. In each of these, the mirror response (i.e. the matching between perception and action) is consistent, suggesting an evolutionary stable core of the trait. Nevertheless, functions, locations, sensorial modalities and developmental trajectories in various MNs kinds are different (both at inter-specific and intra-specific level), so the evolution of MNs as a single trait seems unlikely.

Audio-vocal MNs are the result of *convergent evolution* between songbirds and humans. In both species vocal-learning produces neural circuits devoted to auditory feedback in the motor system, such as neurons activate both during vocalization and listening. However, further analyses of the pathway in which these MNs are located open the possibility that they may represent *factorial homologous* traits, and therefore that audio-vocal MNs may be the result of the expression of similar molecular regulation associated with the sensorimotor regions. Careful research, in line with the pioneering works of neurogenetics in songbirds during recent years [for a review see Scharff and Adam 2013; Fishell and Heintz 2013], is needed to verify this possibility.

Because of their similar cerebral location in frontoparietal circuits across primate species, and because similar neural connections for the control of hand and mouth movements are present in early primates, such as prosimians too, we propose that hand and mouth visuomotor MNs are the result of a *classical homology.* This homology reflects inheritance from a common ancestor who possessed a specific pattern of connectivity between specific cerebral areas giving rise to MNS. Characters might be considered homologous on the basis of their structural correspondence and if the characters themself are more parsimoniously interpreted as having evolved once during phylogeny (Striedter and Northcutt 1991).

Computational models, plus behavioral and neurophysiological evidences related to MNs developmental properties (Keyser and Perrett, 2004; Catmur *et al.* 2007; Arbib and Bonaiuto, 2010; Ferrari et al. 2013) further suggest that primate hand MNs could be considered as homologous at the developmental level as well. In this respect, the phylogeny of MNs can be identified as *developmental* or *epigenetically homologous*, reflecting recurring ontogenetic patterns that tend to reappear reliably at each individual ontogenetic process (Striedter 1998). Emphasizing the developmental similarity of a homologous trait may sound superfluous, but in the animal kingdom it is not rare to find structurally homologous traits that exhibit different developmental trajectories, i.e., originate from different embryonic germ layers (Jenkinson, 1913; Striedter, 1998).

Mouth and hand MNS seem to have different timing in their developmental maturation. The likely involvement of mouth visuomotor mirror responses in neonatal imitation suggests that mouth MNS may emerge more precociously than hand MNS. In macaques, mirror mechanisms related to mouth actions have been detected in the first postnatal days of life, while the observation of hand actions produce activation of sensorimotor regions only after a week of postnatal development (Casile et al. 2011).

Mouth mirror mechanisms may have undergone a stronger selective pressure in the context of the increasing social demands experienced by anthropoid primates about 30 mya, which likely implied a strengthening of mother-infant relationships and face-to-face interactions as instrumental for survival (Dunbar, 2010; Casile et al. 2011; Matsuzawa, 2014). Whether hand MNS develop at a later developmental stage, compared to mouth MNs, because of a different evolutionary history and different environmental demands, requires further research.

Considering the occurrence of different rates of evolutionary changes related to the emergence of various categories of MNs in the different species and in different cerebral regions, we interpreted MNS as the result of mosaic evolution. The mosaic evolution hypothesis posits that in order to understand how the mirror cognitive system emerges, a confluence of multiple mechanisms was necessary, each mechanism having different precursors. Following this hypothesis we suggest different evolutionary trajectories for the various categories of MNs, each with unique adaptations (specific cortical areas, i.e.

premotor, parietal, temporal cortices), exaptations (functional shift observed in phylogeny), environmental challenges, and different timing in developing novelties (i.e. tool responding mirror circuits).

The integration of classical and developmental homology in primate MNS is supposed to synthesize together both the observed developmental plasticity (Heyes 2010; Ferrari et al. 2013) and the phylogenetic continuity (Rizzolatti and Arbib 1998; Casile et al. 2011) of this trait. This is compatible with the hypothesis, mentioned in the introduction, that focuses on the role of epigenetic processes underlying developmental plasticity and canalization processes (Del Giudice et al. 2009; Ferrari et al. 2013) in the development and evolution of primate MNS. As previously explained, in fact, such processes differentially give rise and maintain the considered connectivity patterns in the brain (Dor and Jablonka, 2010; Jablonka, 2012).

The current article not only offer a new perspective which suggest some hypotheses on MNs evolution, but also widen the heuristic potential in predicting the circumstances under which specific variations in MNs activity are expected. Such predictive value is critical to test new hypotheses about MNs activity and its plastic changes, depending on the species, the neuroanatomical substrates, and the ecological niche.

The comparison between human and macaque hand MNs seems to be in line with a case of multiple neural reuse, which gave rise, in the human lineage (and possibly in apes) to the emergence of a specific class of MNs selective for tool actions (i.e. tool – responding MNs). This approach may stimulate more focused investigations on the role of MNs in the evolution of tool – use and associated cognitive traits, such as emulation/imitation of complex manual gestures.

The analysis of early developmental environments of monkey and primate species may provide a guide for future research on perception of mouth gestures and its neural basis, both in primate and prosimian neonates. Finally, the comparison between MNs related to perception and execution of vocalization in songbirds and humans, might pave the way to the validation of interesting evolutionary hypothesis.

Acknowledgments

The work was supported by NIH P01HD064653. We thank Francesco Suman, Elisabeth Simpson, Alessandro Minelli and Richard Moore for their precious suggestions and critics to the work. Figure 2 has been adapted by Pfenning et al. 2014.

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General conclusion

The analysis and interpretation of molecular and neural mechanisms underlying the ontogeny and phylogeny of manual, facial and vocal gestures, is the core of this doctoral work. In particular, the role of epigenetic regulation of gene expression under the temporal influence of various own and others' gestures perception in the development of the mirror neuron system, has been considered as a key factor in the formation of sensorimotor coupling.

Across the manuscripts, it is possible to acknowledge how the concept of epigenetics has been recalled in describing mirror mechanisms. As epigenetics is considered the study of the spatial and temporal control of gene expression in different tissues (Holliday 1999), the account of how mirror neurons develops predictably and functionally in individuals considers how innate mechanisms (i.e. specie – specific rules of genetic regulation in considered tissues) interact with experiences across time and species. Thus, the emergence of mirror neurons during development is interpreted as the organization of the synaptic connections of specific neurons under the influence of 'expected environmental stimuli' (i.e. which belong to the typical social environment of the individual), thus producing noteworthy molecular profile at the nuclear level and identifiable neurophysiological firing pattern.

In trying to identify the key role of inherited components, developmental constraints, timing and variability, some possible scenario of how a mirror neuron system evolved has emerged. The role of epigenetics and transcription processes underlying developmental plasticity and canalization has been used here to describe not only ontogeny, but also the possible phylogeny of mirror mechanisms associated to manual, vocal and facial gestures in primates.

The evolution of mirror neurons has been therefore investigated through the complementary concepts of *plasticity* and *robustness*. If epigenetic regulation of genes expression in specific neurons may account for the interindividual variability recorded in various individual brains, the process of canalization could be used to understand mirror neurons stability and reproducibility across generations and species. Accordingly, throughout the manuscripts, the developmental properties of mirror mechanisms underlying specific mouth and hand gestures have been interpreted as *canalized*, to some extent, similarly to song learning in songbirds or emotion expression in human beings (Gottlieb, 1991; Leppanen & Nelson, 2009). As we have seen, these may be interpreted as cases of *experiential canalization,* a sort of species – specific perception capacity and behavioural responses for which individuals of a given species respond in a characteristic way to certain stimuli (i.e. respond more significantly only to determined patterns of sensory stimulation and not to others) (Gottlieb, 1991), in order to efficiently develop a specific behavioral response. Accordingly, the mirror mechanism associated to facial perception would be a case of developmental sensitivity *only* to environmental factors that are themselves invariant within the organism's typical environment of development (Ariew, 1999).

The fine mechanisms for which these kinds of phenomena occur are still unknown; however, the hypothesis is that during windows of development, such as the neonatal and infant phases, some primate species are attracted by particular biological stimuli and predisposed to react to them in a specific way. Thus, similarly to other canalized traits, experiences with the own body and the caregivers seem to regulate facial and hand gestures coordination in primate newborns, promoting the stabilization of cognitive developments through the regulative control of specific sensorimotor neural structures, such as mirror mechanisms.

It is worth noting that the concept of *experiential canalization* is somehow equivalent to the concept of *sensitive period*. Both processes may be in fact intended as developmental regulated shifts in sensitivity toward various aspects of the environment during an early period of development. They are interpreted as maturational stages in the lifespan of organism, during which the nervous system is more sensitive to specific experiences. Therefore, another crucial concept associated to both experiential (or developmental) canalization and sensitive periods, is *timing*. Implicit in these phenomena, in fact, is the acknowledgement that there are specific times for the organism to receive the appropriate experiences; otherwise, the individual maturation will proceed atypically or problematically.

Several sensitive periods have been identified over time, including the neural mechanisms controlling imprinting in ducks and attachment in many mammals (Lorenz 1937; Bornstein 1989), binocular vision and hearing in some vertebrates (Hubel & Wiesel 1979), and vocal learning in humans and songbirds (Nottehbohm 1992; Bolhius et al. 2010). How these different developmental stages relate to each other's is still poorly understood. Whether specific mechanisms underlie sensitive periods or they are a natural consequence of functional brain development is unknown. However, it seems that high – level cognitive/behavioural skills, like vocal learning or action recognition, involve the integration of many low – level systems. Interestingly, the plasticity in the acquisition of some high – level skills is likely to be the combinatorial result of the relative plasticity of underlying sensorial, motor and sensorimotor systems, along with the developmental interactions among these components. The literature currently available, in fact, suggests that level of plasticity tends to be reduced in low-level sensory systems before it reduces in high-level cognitive systems (Huttenlocher et al. 2002).

The analysis conducted in this Ph.D. work aims to include the development of sensorimotor structures, i.e. mirror neuron system, in the list of sensitive period phenomena. Along with this, it attempts to identify the different factors and mechanisms that are involved in it. In other words, the current work suggests that the development of the mirror neuron system follow the typical processes and characteristics of the sensitive periods. Further, it considers the field of epigenetics crucial to understand how specific properties of neural networks emerge and how plasticity is markedly reduced at some point in development. Nevertheless, how constraints in sensorial processing (i.e. facial preference) emerge during phylogeny and express during ontogeny, the dynamics of opening and closing of various phases of major brain sensitivity and the stabilization of the associated cerebral and behavioural trait are very complex processes, which can be hardly resolved within a single doctoral work, and hopefully will be matter of inquiring for future research.

"Mirror neurons in the tree of life" is a difficult and ambitious project. In proposing an account of mirror neuron development and evolution, it also tries to integrate behavioural, neural and molecular levels for understanding some key properties of sensorimotor cognition. As such, this combined account integrates different cross – disciplinary scientific evidences in a whole theoretical account, providing a comprehensive framework for the explanation of animal behaviour (i.e. multimodal gesture use) throughout a strict focus on the brain. The assumption that justifies this project is that it may be useful for biologists to recognize the theoretical framework within which they conduct their work. The fragmentation of biology into many sub-disciplines, the rapid expansion of data and approaches, and the difficulty of making connections among research results from apparently disconnected areas, may in fact constitute an obstacle to progress in scientific practice and in understanding the functional role of specific neurophysiological mechanisms.

In this respect, it is worth to note that concepts from the field of philosophy of biology have been used to interpret data from neuroscience. Evolutionary theory is a central focus of philosophy of biology, mostly because philosophers agree on Dobzhansky's idea that evolution is a unifying theory of life science (Sober, 1993). As consequence, philosophy of biology may provide useful conceptual tools and instruments to analyse comparative data regarding brain mechanisms. This is the case of the concepts of *canalization*, *niche construction*, classical and *factorial homology* (homology in the expression of similar molecular regulation associated with the development of a specific trait) and *evo – devo* (the field of evolutionary developmental biology, intended as the analysis of developmental variations in evolutionary perspective). Along the manuscripts, each of these terms has been used to give an account of data related to the mirror neuron system.

As for example, the concept of *niche construction* has played a crucial role in the explanation of development and evolution of sensorimotor structures and associated conventional gestural behaviour. Part of the debate between different accounts (genetic *versus* associative) of mirror neurons has focused on the differentially role assigned to learning and social environment. Briefly, some neuroscientists proposed that the macaque and the human mirror system are part of an evolutionary conserved neural mechanism that has been selected during phylogeny for accomplishing high-level cognitive functions, such as action understanding, imitation, mind-reading and language (Rizzolatti & Arbib, 1998; Gallese & Goldman, 1998; Gentilucci & Corballis, 2006). On the contrary, some models mainly addressed the question of the ontogenetic origin of mirror neurons, that are thought to acquire their observation-execution matching properties through a domain-general process of sensorimotor associative and social learning (Cook et al. 2014), thus limiting the possibility that mirror neurons can emerge as part of an evolutionary process finalized to the formation of this population of neurons.

In this respect, the concept of niche construction have helped in proposing a solution to this controversy, highlighting that specific phenotypic traits recognized in different population of individuals not only are affected by natural selection, but also create and maintain specific environments through their activities, which become integral parts of the evolutionary process itself (Odling – Smee et al. 2003). Environmental and social niches are in fact potent evolutionary agents, introducing feedback loops between organisms and environment and new selective forces. This is the case of the niches intended as nursery environment for offspring or for the expression of new behavioural patterns (i.e. tool – use), which in the case of mirror neurons are likely to change and co-evolve with behaviour and brain themselves. Whenever sensorimotor training produces such a profound change, increasing the complexity of cerebral substrates involved in specific tasks and allowing the subjects to perform new cognitive abilities, then these changes could constitute a supplementary source of new variability in evolution along with the environmental interactions that sustain them.

Accordingly, the neonatal sensitivity of anthropoid primates to facial mimicry has been connected to their highly communicative environmental niche. A progressively more demanding social niche may have produced a new selective pressure on individuals that were more efficient in intraspecific communication (Schultz & Dunbar, 2007), favouring in turn the coordination of dyadic facial events also during precocious affiliative and communicative exchanges. Further, in evolutionary terms, the manipulation of tools could have constituted a new and challenging environmental niche as well. This new environment could have led primates to undergo profound changes in specific areas of the brain, producing a brain-derived plasticity via epigenetic phenomena.

In sum, these examples suggest that giving more recognition to theoretical and conceptual frameworks would likely enable scholars of life sciences to make connections between their work and work in other sub-disciplines and, indeed, other fields of science, inspiring the rethinking of assumptions in order to reformulate innovative new research questions. Answering these new questions could in turn lead to new experimental approaches and the birth of new theoretical frameworks.

The potential power of a theoretical account to open new experimental questions is hopefully a characteristic of this doctoral project as well. Such heuristic value is critical to test new hypotheses about mirror neuron system and its plastic changes, depending on the species, the neuroanatomical substrates, and the ecological niche.

Regarding the role of transcriptional regulation in the development of mirror neurons, we may ask what molecular differences exist between standard motor neurons and sensorimotor neurons with mirror properties. In a developmental perspective, we may also ask what neural and molecular differences exist between postnatal and adult mirror neurons and how do such differences affect patterns of mirror neuron discharge.

Regarding the interpretation of mirror neurons as canalized trait, or trait following a specific sensitive period of development, it would be interesting to analyse which social stimuli are able to trigger specific pathways of molecular changes underlying mirror sensorimotor networks. Moreover, identifying the environmental and social conditions that have leaded to a stabilization of the mirror neuron system in different primate species, may highlight aspects related to their evolutionary history.

In the light of the distinction between different types of mirror neurons (hand and mouth visuomotor mirror neurons, tool – responding and audio – vocal mirror responses), a number of interesting research questions may be listed as well. As for example, the comparison between human and macaque hand mirror neuron systems has been interpreted as a case of multiple neural reuse, which gave rise, in the human lineage (and possibly in apes) to the emergence of a specific class of mirror neurons selective for tool actions (i.e. tool – responding mirror neurons). This approach may stimulate more focused investigations on the role of mirror neurons in the evolution of tool – use and associated cognitive traits, such as emulation/imitation of complex manual gestures.

Moreover, the analysis of early developmental environments of monkey and primate species may provide a guide for future research on perception of mouth gestures and its neural basis, both in primate and prosimian neonates. The phenomenon of neonatal imitation and the nascent mirror neuron mechanism may be further matter of inquiring, in order to identify causes and consequences of individual differences in face tracking and facial expressing, through behavioural and neuronal longitudinal studies of newborns, genetic analyses and possibily epigenetic analyses of peripheral tissues (Provencal et al. 2012). Finally, the comparison between MNs related to perception and execution of vocalization in songbirds and humans, might pave the way to the validation of interesting evolutionary hypotheses.

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Acknowledgment

As collaborations and reciprocal exchanges are intrinsic values for research (and for social life in general), this doctoral work would not has been possible without the people that at various levels and extent have provided practical and emotional support, help, ideas and inspiration.

Firstly, I want to mention my supervisor *Pier F. Ferrari*. As I am completely aware that three years of work with me has not always been easy, a special thanks is for him, who with enthusiasm, involvement and competences has helped me to grow. He has been a steady reference point for research, and sometimes also for finding solutions to bureaucratic problems.

I want to devote another special thanks to my external supervisor *Atsushi Iriki*. I have been very lucky to know him, as he is one of the more original scientists I have ever had the pleasure to meet. He has always offered me inspiration, challenges and new possibilities to explore.

Further, I want to thank *Lynne Murray*, *Alessandro Minelli*, *Kazuo Okanoya*, *Luca Bonini* and *Marzio Gerbella,* who have always been available to give me suggestions, critical comments, materials and interesting analyses of my work. Their contributions have been precious and beneficial.

It is hard not to mention *Marianna Ambrosecchia*, *Fabrizia Festante*, *Ylenia Nicolini*, *Gino Coudè*, *Marco Bimbi*, *Katrin Heinman, Sebo Uithol, Doriana De Marco*, *Elisa De Stefani, Maria Teresa Sestito, Marta Calbi* and *Monica Angelini* as well. Each at his/her own manner has contributed to make better my life in the Parma Department of Neuroscience, with interesting discussions and exchanges, but also simply with their presence.

Life in research has also given me the occasions to know interesting and inspiring people, such as *Irene Berra*, *Francesco Suman*, *Holly Raison, Fabrizio Mafessoni* and *Robin Chao*. The friendship and esteem that I harbour for them have constituted the basis for rich exchanges and new collaborations.

Last but not least, a great thanks to my family, to my father for his quiet and comforting support, to my mother for her warm vicinity and practical help, and to my beloved brother and sister for being always available to pay attention to me.

Finally, thanks to all the friends and people that I encounter on my road and contribute to make my life happy and satisfying.