Original article

☐ Just how consistent is the mirror neuron system paradigm?

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SUMMARY: *The purpose of this work is to challenge the existence of mirror neurons and the so-called mirror effect by conducting a detailed analysis of some of the experimental measures used on monkeys that led to the formulation of the mirror neuron system paradigm, as well as the non-invasive experiments since performed on humans. It is the Author's conjecture that once the literature often cited in support of mirror neuron theory has been carefully examined, numerous lacunae become evident, which, together with a modest dose of common sense, would seem to cast considerable doubt not only on the science behind the evidence, but also the logic behind the paradigm itself. First and foremost, the experiments carried out by many mirror neuron system theory supporters have been performed in an artificial laboratory context in which the participants were measured for, and ascribed, particular properties that are as yet unverifiable and not yet fully understood. This appears to have opened the door to an "optimistic" interpretation of the data, particularly concerning complex phenomena such as empathy, imitation, etc., that do not yet share unified semantics. Furthermore, techniques used to measure such properties, for instance functional magnetic resonance imaging, suffer from gross limitations, and there are some notable discrepancies in terms of timing, among other things. Hence the results reported so far, and the mirror theory itself, should be treated with extreme caution, and the current trend in applying this hypothesis to real-world treatment protocols, which is currently underway in rehabilitation and autism spectrum disorder should be halted or even reversed until such time as the situation is clarified.*

KEY WORDS: *Action understanding, Autism, Mirror Neuron System.*

☐ INTRODUCTION

By 2008, several hundred scientific articles had been published on the subject of mirror neurons (Figure 1) and catalogued in PubMed. Some 20% were written by the Authors of the paradigm itself and associated research teams, and the majority of the remaining 80% were produced by Authors who agree that there is indeed such a thing as the mirror neuron system. However, a large minority of works have been prof-fered by MNS sceptics and those who refute the paradigm entirely, and copious literature has been published on the function of neurons, and the brain in general, with no particular focus on the mirror effect[11,30]. Since 2008, scientific output on the topic has greatly increased, but the graph shown in Figure 1 is still a valid representation of the current trend.

The articles produced can be classified according to type, and of the many documents considered by the Authors of this paper, more than 20% were reviews.

LIST OF ACRONYMS AND ABBREVIATIONS: ASD = Autism Spectrum Disorder; **BOLD** = Blood Oxygenation Level Dependent; **EEG** = ElectroEncephaloGraphy; **fMRI** = functional Magnetic Resonance Imaging; **MEG** = MagnetoEncephaloGraphy; **MN** = Mirror Neuron; **MNS** = Mirror Neuron System; **PET** = Positron Emission Tomography.

As the graph shows, only 80% consisted of experimental studies and those of a speculative nature, such as some of the articles on language[2]. There are also some notable studies that predate the formulation of the paradigm[12,23], and others related to but not directly concerned with the topic[33,40,54], that need to be taken into consideration if a well-rounded picture of the situation is to be formed.

Leaving aside all the speculative articles and reviews on the subject, the experimental studies can be grouped into two categories: those carried out on monkeys and those, less invasive, performed on humans. As is evident from the graph, the latter far outweigh the former in terms of number, although, at best, they can only provide indirect evidence of the MNS in humans, as their non-invasiveness (fMRI, PET, EEG, etc.) means that they lean heavily on purported similarities between simian and human brains. Although invasive experiments have been performed on monkeys, *PubMed* provides few examples, and instead contains a predominance of speculative studies that merely present reflections on the subject, their arguments being drawn from prior experimental studies or reviews.

Figure 1 shows the pseudo-exponential growth of the literature in question. This is primarily due to the expectations of the scientific community, which no longer feels comfortable with the models of simi-unculus or homunculus, and has leapt at the chance to explore the MNS, apparently a new systematic skeleton key (a new 'paradigm' to use the language of Kuhn) able to unlock just about every door, from autism[3] to empathy[18], from the understanding and processing of speech[42], to sexual preference[39], to say nothing of general art interpretation[17,31], schizophrenia[19], criminality[1], mind reading[22] and "action understanding"[45], to cite just a few examples.

The cornerstone of all this literature, and the MNS paradigm itself, dates back to a study published in 1992 (di Pellegrino et al.)[12] in which the Authors report that they have identified neurons in the monkey premotor cortex that discharge both when the animal executes a particular action and when it observes another individual performing the same action. In 1996, following an experimental study by Rizzolatti et al.[45] the term mirror neurons was coined to describe these cells, which were ascribed the ability of "action understanding" i.e., to help us "to understand the action of others 'from the inside'."

Testament to the popularity of such an appealing idea, rather than seeking to confirm or refute such findings, or those reported by Gallese et al. in 1996[20], subsequent experiments on monkeys were designed to open new areas of research[33,40,54]. Thus, the MNS paradigm was created in 1996, and consolidated thanks to continual citations and ample reviews rather than convincing experimental data. Indeed, our *PubMed* search revealed no further works by di Pellegrino et al. on mirror neurons, though their 1992 data is still the touchpaper in the continuing debate on the topic.

Anatomical parallelism between monkey and human brains (F5 and Broca's area) have favoured experiments designed essentially to translate the paradigm from monkeys to humans by means of non-invasive techniques (EEG, fRMI, etc.), with little effort made to repudiate the original simian findings through hard data (according to Popper's falsifiability model) or formulate alternative paradigms. It is no mere coincidence that innumerable papers begin with a phrase akin to: 'Mirror neurons have been discovered in monkey brains...,' as they merely rehash the original studies. Therefore figure 1 of a review published by *Nature Clinical Practice Neurology* in 2009[44], echoes that published in previous articles (Rizzolati et al., *Current Opinion in Neurobiology* 2008[43] and Fabbri-Destro et al., *Physiology* 2008[13]), which in turn mimic that published in the original experimental study by di Pellegrino et al.[12] (their figures 2 and 3). Another loan, figure 2 of Iacoboni et al., *Nature Reviews Neuroscience* 2006[27], was borrowed from Rizzolati et al., *Nature Reviews Neuroscience* 2001[46], but was first published in *Cognitive Brain Research* in 1996 by Rizzolatti et al.[45]. Similarly, to analyse the content of the paradigm and its various implications, with reference to the interpretative aspects of autism, Rizzolati et al. (*Nature Clinical Practice Neurology*, 2009)[44] refer to the study by Cattaneo et al. that appeared in *Proceedings of the National Academy of Sciences of the USA* 2007[7]. In this context, it is also interesting to analyse the paper written by Iacoboni et al. that appeared in *Nature Reviews Neuroscience* 2006[27].
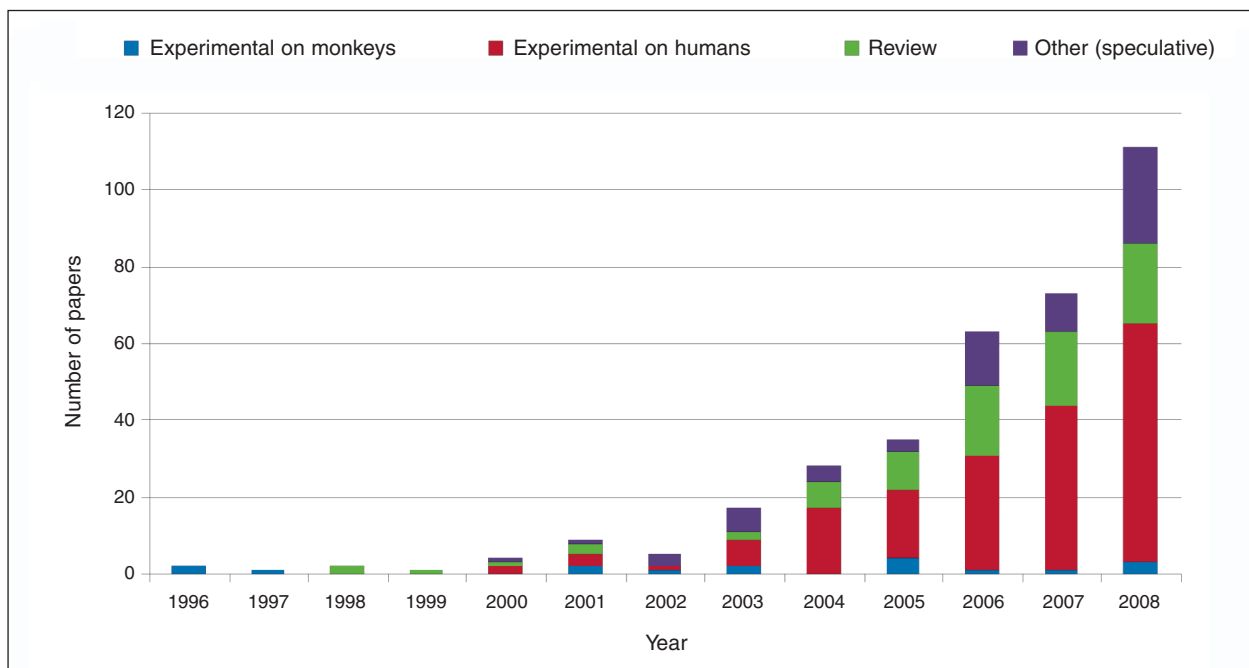
Figure 1. The rising trend in MN literature. The number of papers retrieved from *PubMed* that include the terms: "mirror neuron," "mirror neurons," "mirror neuron system" or "mirror system" in their title, abstract or keywords, as a function of the year of publication (since 1996). Note: this exponential trend is still maintained.

It is apparent then, that discussion of the paradigm cannot avoid harking back to the foundation studies, which, as we will see, would benefit from detailed examination. Indeed, although many advanced fMRI studies have been conducted in this field, a large proportion of this research strongly rests on the foundations of the discovery of the MNS in monkeys, taken as incontrovertible fact, which is debatable, and therefore indirectly gives rise to additional elements of doubt[21,28,38,48]. The link that has been forged between experiments on monkeys and those on humans is tenuous to say the least, and the purported existence of mirror neurons in humans is therefore equally shaky.

Is the MNS paradigm consistent in the light of fMRI? And, more importantly, is fMRI the right tool for measuring the activity of mirror neurons? Although it should be stressed that an individual who takes part in an fMRI exam does not develop significant movements, such studies require prolonged examination of the area/brain region in question to obtain useful data. Moreover, fMRI involves recording haemodynamics caused by the neural network, which are, at best, indirectly connected to the experiment. Haemodynamic activity caused by the discharge of a few neural elements cannot be sensed with the haemodynamic variation effect of the BOLD signal, which is the product of variations in the deoxyhaemoglobin concentration in the vasal network, mainly on the prevenular side. In these experiments, electro-neural activity from the cerebral convolution is correlated to the production of a haemodynamic signal that features time delays in the order of seconds and is topographically located in the sulcus between two adjacent convolutions. With reference to Figure 2A, which of the two convolutions is being measured? Figures B and C show the complexity of the 'network' in question, a complexity that provides ample opportunity for interference.

Although faint hope seems to be provided by the work of Mukamel et al.[32], based on the detection of neuronal activity on single cells in humans, it appears that the proponents of the MNS theory continue to underestimate the importance of assessing the temporal relationship (delay, during or in advance) between an observed gesture and the neuronal activity of the observer. The MNS experiments carried out using fMRI have been subject to review in *Mirror neurons in humans: consisting or confounding evidence?* by Turella et al.[52], which, like others, attempts to test the presumed solidity of the paradigm. This concludes that recent studies based on fMRI adaptation protocol to search for mirror neurons in
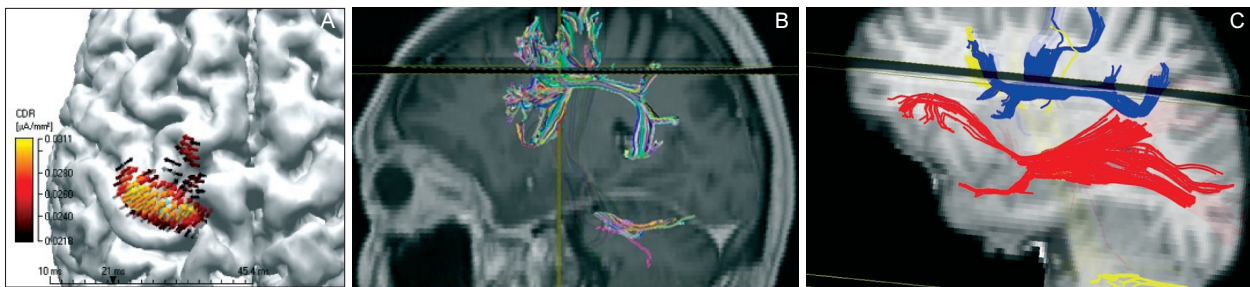
Figure 2. An fMRI study of the human brain. A. Position of the BOLD signal with respect to the convolutions (Examination carried out with Siemens Avanto, fMRI 1.5 Tesla and post-processing with Curry 6.0). B. The cross indicates the exact position of the stimulation electrode obtained during a surgical operation. The image, anatomical and not functional, shows the course of connecting fibres in cerebral white matter. The image was obtained by magnetic resonance. The fractional anisotropy was recorded and then diffusion tensor imaging was performed. The course of the fibres is located (as an MR signal vector that evolves in a specific direction XYZ) between a departure area and an arrival area. According to the direction taken by the fibres, the signal is codified in different colours (green latero-lateral, blue antero-posterior, etc.). C. The colour traces reach the cortex and demonstrate the potential for interference and the complexity of the neural network (*Internal documentation of the Industrial Bioengineering Lab, University of Udine*).

humans have obtained negative or controversial results. Even the representation of results through histograms, obtained as the sum of repeated individual events, without taking into account the characteristics of each individual event (phase, intensity, persistence, etc.), is inadequate, because it cancels out the specificity of each individual neuronal response concerning the phenomenon that is under investigations. Indeed, according to Logothetis[29], who also sets out the limitations of fMRI[9], the prevailing sense is that one should return to examine the initial work, that is, the experiments on monkeys, before proceeding with human experiments[20,42,53].

These considerations will be discussed in more detail below, in the section: "What was actually seen in the original experiments?"[35], in which the Authors start from a paper, published in 2008[36] that focused on the analysis of measurements of individual neurons in monkeys, conjecturing that the seminal experiments on monkeys have been given their appropriate weight within the scientific community.

☐ IS THE MIRROR NEURON SYSTEM PARADIGM CONSISTENT IN TERMS OF THE METHODS OF EXECUTING MOTOR ACTIONS?

No one can fail to notice the behaviour typical of an adult in the early stages of educating a child. With a look, the adult gets ready to 'help' the child, repeating/following, perhaps mentally anticipating the sequences that the child is expected to carry out. In this situation,

a hypothetical recording of neural activity would doubtless register delayed or synchronized electrical activity triggered by this anticipation (preparation of the motor action) in one individual with respect to the other. It is therefore necessary to examine the motor actions of individuals on an appropriate timescale and, in discussing neural circuits, the study of a hypothetical 'mirror' event should provide an examination of every single action, real or 'virtual', and of the overall action, with an equally detailed analysis of the times taken to complete these actions.

Boxers are a helpful example to use because all the action takes place within the reach of their arms, and attack and defensive actions, including those involving trunk movement, occur within 200 ms. The boxer on the defensive has to move to ward off his opponent's fist (note the still arms at time 0 in Figure 3). It is not by mirroring his opponent's gesture that he is able to avoid the blow - perhaps it is an expression on the opponent's face or a gesture by his body, the position of his pelvis or his feet, the action he had previously performed, or a combination of all of these and other factors that give the game away, but in any case, he is able to anticipate his opponent's next move within a very short time frame[5,36,37]. In short, there is no "embodied simulation", as the process would be too slow to allow a boxer to defend himself. Instead there is a prediction of action-intention of the opponent, stemming from previous experience of tactical and strategic schemes, conferring the ability to anticipate what is coming.

In the case of observed actions, if a mirror effect existed in our brain, "regularities" should be expected,
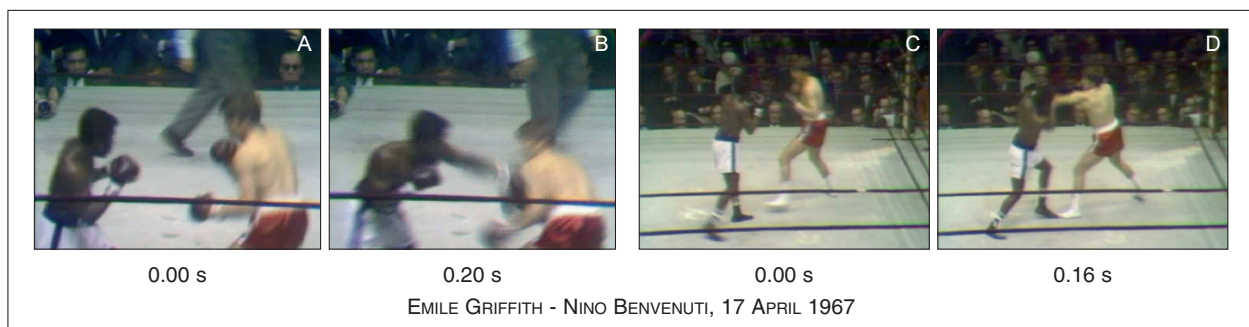
Figure 3. Reaction times. Boxer action and (anticipated) reaction (A and C still punch, B and D completed actions).

taking place between the experimenter's gesture and the "mirror" neuron firing, with delays compatible to neural circuitry. If, on the other hand, the "virtual" event develops in one's (or a monkey's) neurons in advance with respect to the action proposed by the opponent (or experimenter, for instance in the act of grasping[6]), the activity of such neurons could not reasonably be said to "mirror" the action. Thus, the MNS paradigm would fail. This can be summarized by the schematic reported in the box on the next page. Bearing in mind the works cited in the introduction, it is worthwhile calculating the presumed or assumable temporal phase displacement between the executed action and the observed action. In some cases it is easy to estimate the neuronal timing without using gruesome experiments, as is clear from Figure 6. Let us take another example from the world of sports, i.e., sprinters. Even in the 100 m or 200 m event, their times are fairly analogous to those of boxers. In the "Ready, Set" phase, the runner prepares for action and, as soon as the gunshot is perceived (i.e., a pre-formed neuromuscular chain), starts off in a specific direction (pre-ordered). Reaction times recorded during a representative race for several athletes are: M. Johnson 0.161 s, A. Powell 0.134 s, and U. Bolt 0.165 s. Such values take into account time delays caused by sensory receptors, neural transmission to the cortex, information recognition (in this case the gunshot), neural transmission to muscles, muscle latency and time of muscle activation. The neuron system, in this case of an "audio-motor" effect, starts firing about 30 ms - or perhaps less - after the gunshot, and the "visual-motor" effects have the same delays[26]. Hence, under experimental conditions, an MN should start firing with a similar time delay following the experimenter's gesture towards the target.

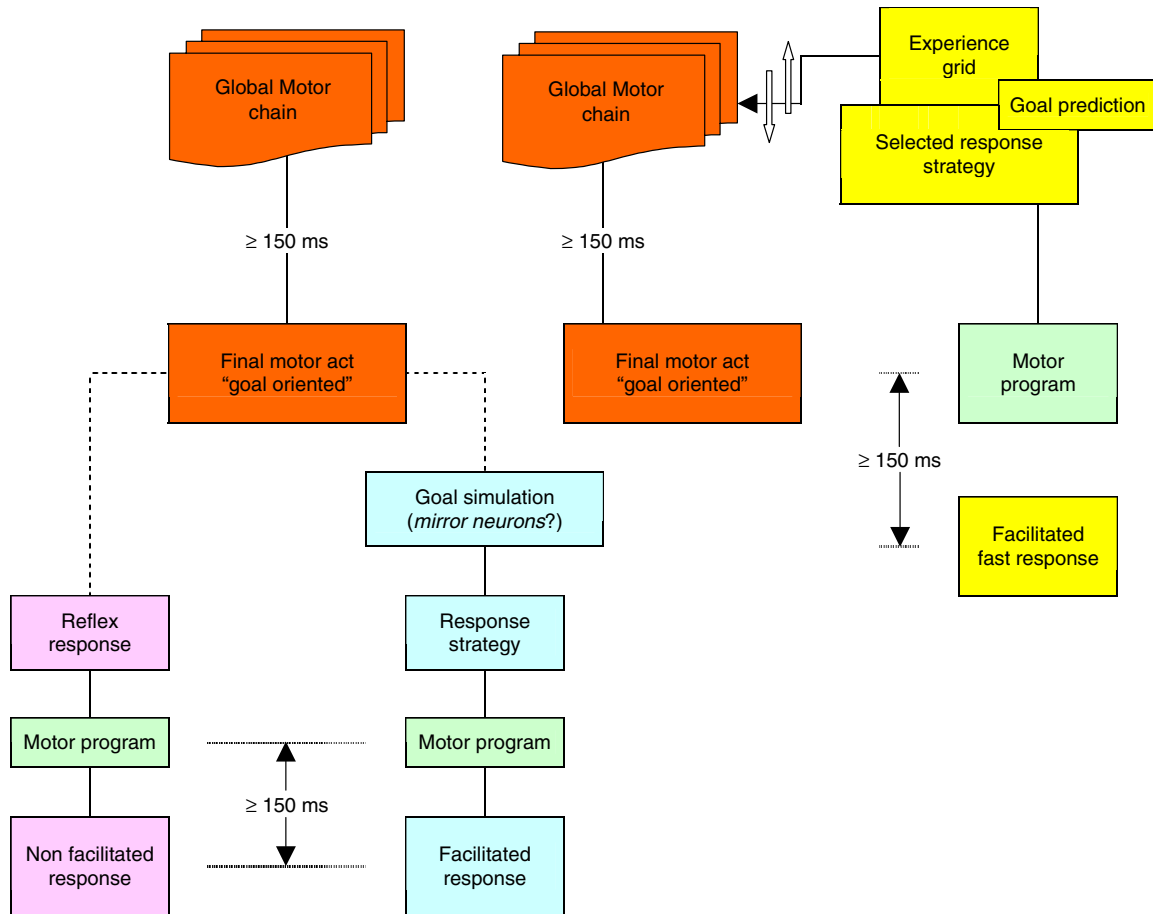Preparation and anticipation are the result of ex-

perience, as well as, in the case of competitive events, interpretation of the opponent's movements or expressions linked to the expected event that is yet to occur. If this were not true, only time delays between perceived action and physical reaction, i.e., conventional psycho-technical reaction times, would apply, as the sportsperson, for instance, would know that they have to perform a certain action but would not know when. In this scenario, a goalkeeper would have little chance of saving a penalty, as reaction times range from 300-350 ms to 600-1000 ms in normal people. However, a good goalkeeper, after studying the characteristics of the penalty taker, will start moving at least 300 ms before the kick. Hence, any possible save is the result of calculation, not of unconscious anticipation.

As previously specified, the experiment recalled in figure 1 of Rizzolati et al., *Nature Clinical Practice Neurology* 2009[44] and reported here below in Figure 4, is a foundation upon which all others have been based, in particular those conducted in 1996 by Rizzolatti et al[45] and Gallese et al[20]. These experiments on monkeys need to be re-examined, and if the measurable times are found to be highly inconsistent, the existence of non-mirror collateral events linked to information elaboration/interpretation, competition for food, "virtual" motion anticipation, etc., should be considered.

☐ WHAT WAS ACTUALLY SEEN IN THE ORIGINAL EXPERIMENTS?

To look at these seminal studies more closely, the experiments concerning two neurons, U481 and U483, reported in the 1992 work by di Pellegrino et al.[12] were analysed. One of these neurons, U483 has
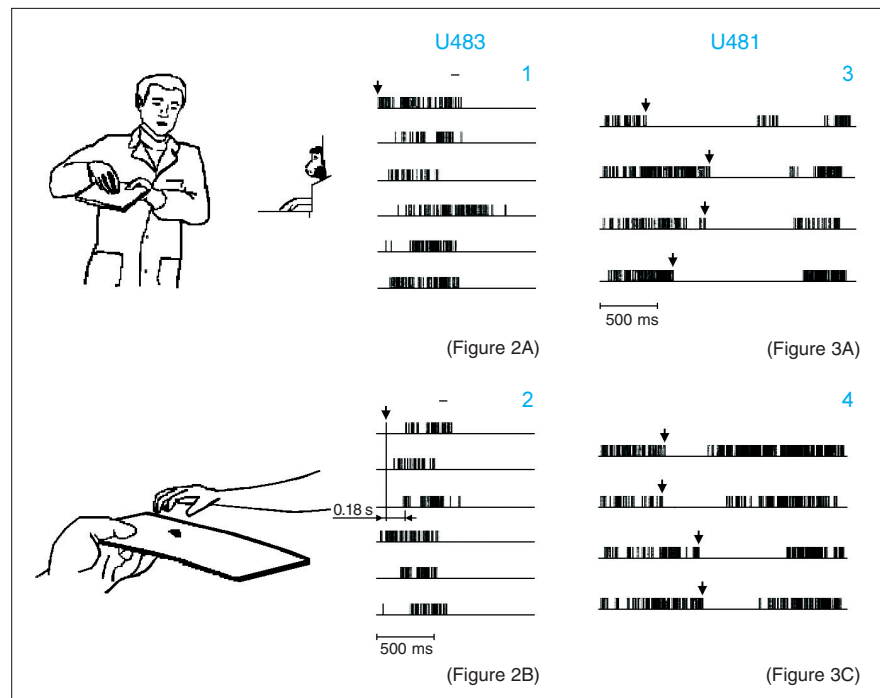
**VARIOUS PATTERNS OF RESPONSE
TO OBSERVED ACTIONS AND THEIR TIMING**

Global Motor chain

Global Motor chain

Experience grid

Goal prediction

Selected response strategy

≥ 150 ms

≥ 150 ms

Final motor act "goal oriented"

Final motor act "goal oriented"

Motor program

≥ 150 ms

Goal simulation (*mirror neurons*?)

Facilitated fast response

Reflex response

Response strategy

Motor program

Motor program

≥ 150 ms

Non facilitated response

Facilitated response

Understanding action means interpreting what another intends to do and being prepared to react accordingly. In a variety of real-life situations, to react appropriately and advantageously, you need to understand the purpose of an observed action well before it develops. It is obvious that the brain of any living organism, when presented with an external trigger, activates mechanisms to interpret the perceived event, but it is also obvious that the brain, geared for survival, provides anticipative dynamics within the "learning-operating" mechanism even between non-cospecific subjects, e.g., a predator studies signs of escape in the prey it is intending to intercept. This signifies assessing/ understanding the intended action of the other by interpreting "precursors" of the action even before it begins to develop (right side of the schematic). To do so it is also clear it will engage the areas of the brain where past experiences that might be useful for the interpretation are stored. This, however, has nothing to do with *embodied simulation* (*left side of the schematic*), which instead implies, according with the MNS theory, the use of the same neurons[12,20,45] that are used during execution of an action similar or equivalent to the one observed.

Schilbach[49] has pointed out that the monkey experiments used to bolster the idea of action understanding were conducted in static and artificial contexts. If you consider the need to understand the action chain (action sequences) and immediately react to the actions of others in cooperative or competitive contexts, but, considering the other as a "second person," you can see how the supposed embodied-simulation is a weak mechanism[21], and redundant, if not a hindrance.

Figure 4. Premotor neurons u483 and u481, as described in the article by di Pellegrino et al. (*Exp Brain Res*, 1992)[12]. Examples of a unit selectively discharging: 4.1 and 4.3 as the monkey observes of the grasping movements made by the experimenter (original figure 2A and 3B) 4.2 and 4.4 as the monkey performs grasping movements (original figure 2B and 3C).

also been studied in other experimental works[13,44]. Looking at Figure 4 (left panel), we can see that this neuron starts firing as soon as the experimenter starts to perform the grasping action, then it stops and restarts after the animal itself begins grasping (original figure 2A and 2B of di Pellegrino et al., 1992[12]). If we examine the time delay from the start of the experimenter's action (vertical, black arrow) and the start of firing, this amounts to a minimum of 10-20 ms and a maximum of 170-180 ms. If the former value is consistent with "neurological" times, the latter is consistent with the activity of processing information by the network containing the neuron. Taken as an average, the time delay is 105 ms. The duration of firing during the monkey's observation goes from 0.42 s to 0.98 s, that is the experimenter's moving change in velocity. The animal's firing times during execution range from 0.31 s to 0.5 s.

The neuron labelled U481, whose behaviour in such circumstances is described on the right in Figure 4 (original figure 3A and 3C in Pellegrino et al., 1992[12]), was examined. As we can see, U481 is always active but interrupts its activity, i.e., becomes silent, exactly at the instant of the onset of grasping, either in the experimenter or in the animal. Curiously, there is no delay, not even 1 ms. This neuron, operating in off-on logic "apparently" in phase with the event, was not used as a trigger signal or examined in more detail,

but was nevertheless cited in future works. However, the function of such a neuron is not merely confined to on-off logic, it also provides other information. Furthermore, the work in question[12] does not report the gesture measurements or the motor acts performed, although an optoelectronic system for kinematic data acquisition (ELITE: ELaboratore di Immagini TEevisive, TV image processing system, BTS, Garbagnate Milanese, Italy) was used. It is reported that only the animal was wired up. In order to provide a more complete picture, the experimenter's arm should also have been subjected to such recordings, as it provides that which the neuron apparently mirrors (with delays ranging from 20-30 ms). This appears to be a rather large flaw in the method, and was only rectified in later works, albeit not in the same kind of experiments[53].

Although some Authors may not feel the need to look for a univocal timing relationship between the gesture observed and the neuronal response, we decided to explore this issue further, focussing our investigations on other seminal experiments. See Figure 5: visual and motor responses of a grasping mirror neuron, a reproduction of both figure 1A from the work of Gallese et al., *Brain* 1996[20], and figure 2A from Rizzolatti et al., *Cognitive Brain Research* 1996[45] (also reported in *Nature Reviews Neuroscience* (2001)[46] as figure 1A). In these figures, the neuronal
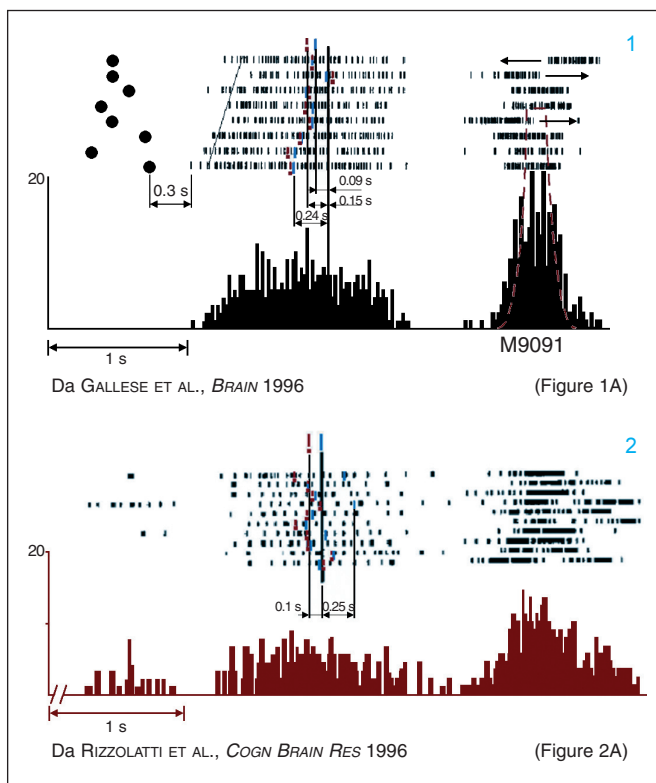
Figure 5. Visual and motor responses of a "grasping" mirror neuron. A tray with food is presented to the monkey, the experimenter grasps a piece of food with his hand and then moves the tray with the food towards the monkey, who makes the grasping movement toward the food. The figures shows the action potentials of the neuron recorded over time for each of the actions: the neuron discharges during experimenter and monkey grasping, but in contrast the neuron ceases to fire when the food is presented or moved toward the monkey. In the upper part of the picture (raster plot), the vertical line across is the moment at which the experimenter touches the food; in the lower part of the picture, (histogram) we can see the frequency of the neuron's instantaneous firing, calculated in intervals (bin) of 20 ms each. The y axes represent spikes/bin and the x axes represent time. The start of the trials is represented by a spot in bold type. Figure 5.1 was taken from figure 1A of Gallese et al., *Brain* 1996[20], and Figure 5.2 from figure 2A of Rizzolatti et al., *Cognitive Brain Research* 1996[45].

the aligning segment - the neuron seems to possess "improving capabilities". Moreover the neuron in the trial in question (Figure 5), as will be discussed in more detail below, seem to complete the "virtual" precision grip around 0.25 s before the experimenter, that is, the monkey anticipates the experimenter's actions. Every single recording of the activity relative to the neuron in Figure 5.1 was considered, both in number of firings and in temporal distance between the first and last registered firing, and it was placed in relation with the vertical segment that aligns the achievements of the goal by the experimenter. The median of firings is indicated in red, and the average time in blue. According to these calculations, the global average time and global average firing is anticipated by roughly 100 ms with respect to the alignment segment with a fixed maximum value of about 240 ms, indicating that the neuron had started firing even before the experimenter's movement. If this is the case, how can it be described as a "mirror"? Indeed, if the neuron anticipates the action, we cannot speak about the "imitation" paradigm. At any rate, the experiment seems to deal with repetitive, rather than improvised behaviour, which would influence or frighten the animal and would, therefore, interfere with the response.

To suggest the validity of using the average as an instrument for measuring the phenomenon in question, and therefore for re-classifying the results of di Pellegrino et al., *Experimental Brain Research* 1992[12] and Galles et al., *Brain* 1996[20], there is a study that records a neuron's times of activity before, during and after a generic exercise[14]. It also reports a - rather grisly - locking device used on the animals, and the component included in the brain case used as support for the measuring system with microelectrodes (Figure 6).

Moving on to a similar comparison, Figure 7 (a reproduction of both figure 1B from the work of Gallese et al., *Brain* 1996[20], and figure 2B from Rizzolatti et al., *Cognitive Brain Research* 1996[45]), it is evident that the signal relative to the first phase of the exercise is relatively weak, perhaps because the exercise was complicated by the use of the pliers to reach the food. The firing times measured on the available data, in the case of the animal's simple

activity is measured during the action of food being grasped from a tray.

When the tray with food is offered to the monkey, the neuron does not fire[45]. If it does not fire, we can deduce that the neuron is of the same family/class as the U483 neuron[12], and should start firing on average around 100 ms from the start of the action, or even 10-30 ms (mirror). This can only occur if the grasping action is begun during the presentation of the tray of food, that is, very quickly (Figure 5). However, in the work it is stated that the presentation of the food on the tray comes first, to show that the neuron does not fire. In Figure 5.1 we can observe the "tendency" (shift) of
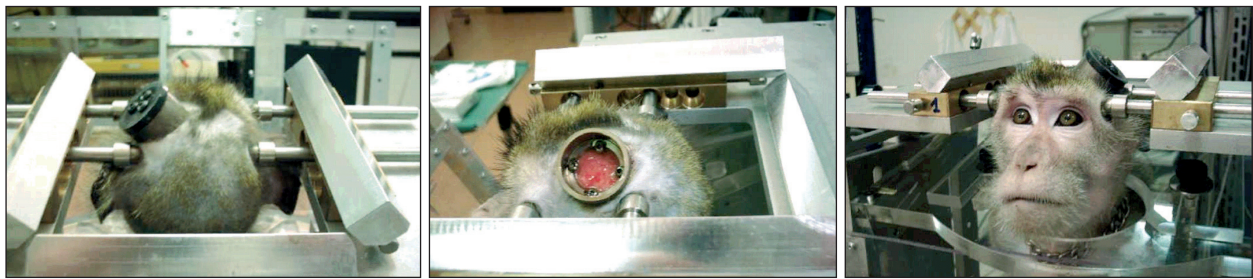
Figure 6. Experimental Apparatus. Common apparatus used for experiments on monkeys (detail from Fadiga, 2004[14]).

observation, goes from a minimum of around 0.2 s to around 1.6 s. We will return to the intensity and quality of the signal found in this part of the experiment later, but for the mean time suffice it to say that the single trials are characterized by irregularities in the positioning of the individual averages. Moreover, Figure 7.1 is incomplete on the right side, making it impossible to determine the firing times of the monkeys' precision grip. Nevertheless the times appear to be substantially greater than those pertaining to the same part of the exercise reported in Figure 5.1.

Among the various experiments documented in Gallese et al., *Brain* 1996[20], there is one regarding the execution of the experimenter's precision grip with the food placed on a stylus (a kind of chopstick) rather than in a tray or manipulated with pliers. This picture is not reported here, but in the original the hypothetical mirror neuron shows intense activity but no regularity of firings with respect to the aligning segment (the exper-imenter reaching the food), with an average delay even as great as 0.5 s. This value suggests additional neural activity of the animal required to understand the complex setting in which the experimenter's gesture takes shape. If mirroring were taking place, this neuron should synchronize with the movement, this being a normal action of grasping food. In short, the first part of exercise 1B should at least reappear in the conclusive trials without showing striking irregularities and delays[20].

Further data in the literature support the pre-vious arguments. Take Figure 5.2, which re-cords the response to an exercise identical to that in Fig. 5.1[20,45]. The average time is posi-tioned approximately on the vertical segment,

which indicates the attainment of the target. Once again, the firing average is anticipated with respect to the alignment - two identical experiments but with different averages, that is, only similar behaviours.

The neuron examined Figure 7.2 does not respond in any way to the action of the experimenter holding a pair of pliers[45]. However, in a similar experiment in Figure 7.1 there was a signal detected, and in some of
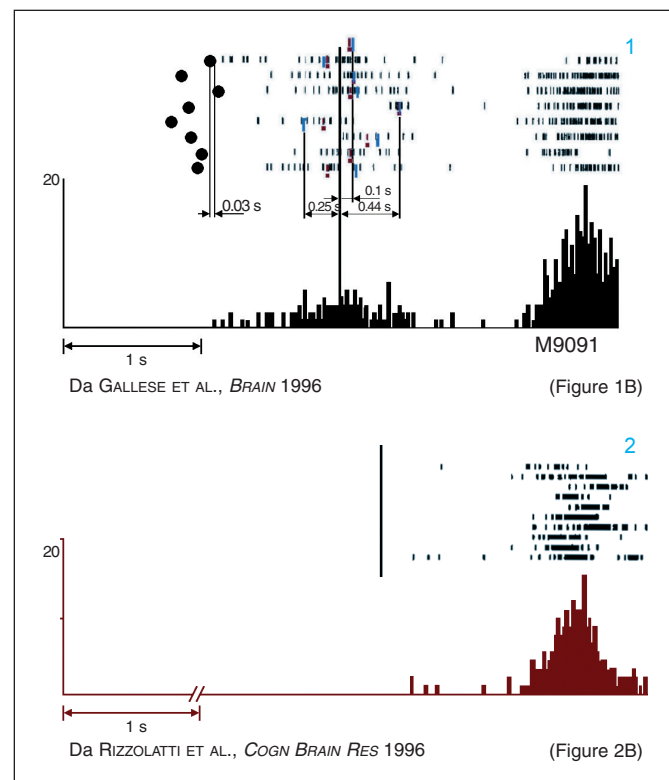

Figure 7. Visual and motor responses of a grasping mirror neuron. The experiment shown is the same as that in Figure 5, except the food is grasped with pliers rather than the hand. Figure 7.1 is taken from figure 1B of Gallese et al., *Brain* 1996[20], and Figure 5.2 from figure 2B of Rizzolatti et al., *Cognitive Brain Research* 1996[45].

the trials it was even strong. The intensity of the firings is greater in the first part of the experiment with the pliers than when the experimenter grasped the food with his hand (Figure 5.2). This being the case, how would the histograms have changed if further trial recordings had been added? Would the same result be illustrated in Rizzolatti et al., *Cognitive Brain Research* 1996[45], and other subsequent works[15]?

What is more, as Figures 5 and 7 are largely approximate, it is worth making the following observations. The measurements are aligned with the attainment of the target by the experimenter; therefore the histograms that relate to the monkey's grasp are the product of sums of out-of-synch events. The correct histogram for the animal's grip would undoubtedly be different from that shown in the figures - certainly tighter and of greater magnitude. Moreover, on this occasion too, by aligning the experimenters action with the monkey's reaction (see the horizontal arrows in Figure 5.1 and the slope over the histogram on the right), a further difference is generated, both quantitative and qualitative, between the two histograms in Figure 5, the two histograms in Figure 7, and so on.

The control experiments are also worth mentioning. In original figure 11 of Gallese et al., *Brain* 1996[20], EMG experiments are reported wherein one mouth and three hand muscles of the monkey were wired up, though no other muscles (for example, the arm), even though the eventual motor action would evolve in a proximo-distal direction. Plus, once again, to study the mirror neuron effect, in addition to the monkey's neuron and forearm, the experimenter should have been monitored in a similar fashion. Once again, in Rizzolati et al., *Cognitive Brain Research* 1996[45], the motion analysis system ELITE is discussed and applied to the animal, as in di Pellegrino's study (1992)[12], but there are still no precise checks of the measurements obtained.

Also somewhat perplexing is the interpretation of the results of the grasp-to-eat and grasp-to-place experiments, shown in figure 2 of the study by Rizzolati et al., *Nature Clinical Practice Neurology* 2009[44] retrieved from Fogassi et al. (2005)[16]. The statement "this coding implies that when the monkey observes grasping done by another, it is able to predict, on the basis of contextual cues (e.g., repetition, presence of specific objects), what will be in the individuals next motor act. In other words, the monkey is able to understand the intentions behind the observed motor act"[44], is far from convincing. Is it that the neuron simply reacts because the monkey sees a different movement, or does it react to the preparation of diverse kinds of actions? This brings to mind the boxer's action shown in our Figure 2, and events connected to the recognition of features or movements (in particular facial), which are dominated by retinal pre-treatment (30 ms according to Honey et al.[26]). Have the facial expressions of the experimenters been classified or 'masked' to avoid the risk of interference due to visual communication? In this regard it is worthwhile remembering an event in 1907, regarding the comparative biologist and psychologist O. Pfungst and the horse *Clever Hans*[50], who appeared to know how to answer complex questions. O. Pfungst effectively debunked this myth when he discovered that Clever Hans simply interpreted the gestures of the observer, thereby providing a notable example of methodological paradigm of experimental fallacy.

At this point, harking back to what is written in the introduction, it is useful to note that in Rizzolatti and Sinigaglia, *Mirrors in the brain*, Oxford University Press 2008[47], some figures (figures 5.9 and 5.10), supposedly representing the brain activity recorded by fMRI in different conditions (humans *vs*. humans, *vs*. monkey and *vs*. dogs), are different to those first reported by Buccino et al. in the *Journal of Cognitive Neuroscience* 2004[4]: some red-yellow areas have been modified, and some areas, specifically the those related to vision, have been made less evident. As a consequence, it may appear to the average reader that they provide better qualitative support for the MNS theory than the original figures (see our Figure 8). These images were meant to illustrate how the comprehension of another subject's motor acts (i.e., the movement of the lips of the observed subject) is high between humans, lower when a human observes a monkey, and very poor when a human observes a dog. In other words the results indicate a monotonically decreasing function, the more the observed subject differs from the observer[34]. In a previous work[36] we expressed our doubts regarding these results, and a recent fMRI study in which "dog experts' brains distinguish socially relevant body postures similarly in dogs and humans" confirms that such doubts are reasonable[28].

□ **AUTISM AND MOTOR ACTS**

MNS theory has been extended to cover conditions such as autism. Although it is a seductive hypothesis[24]
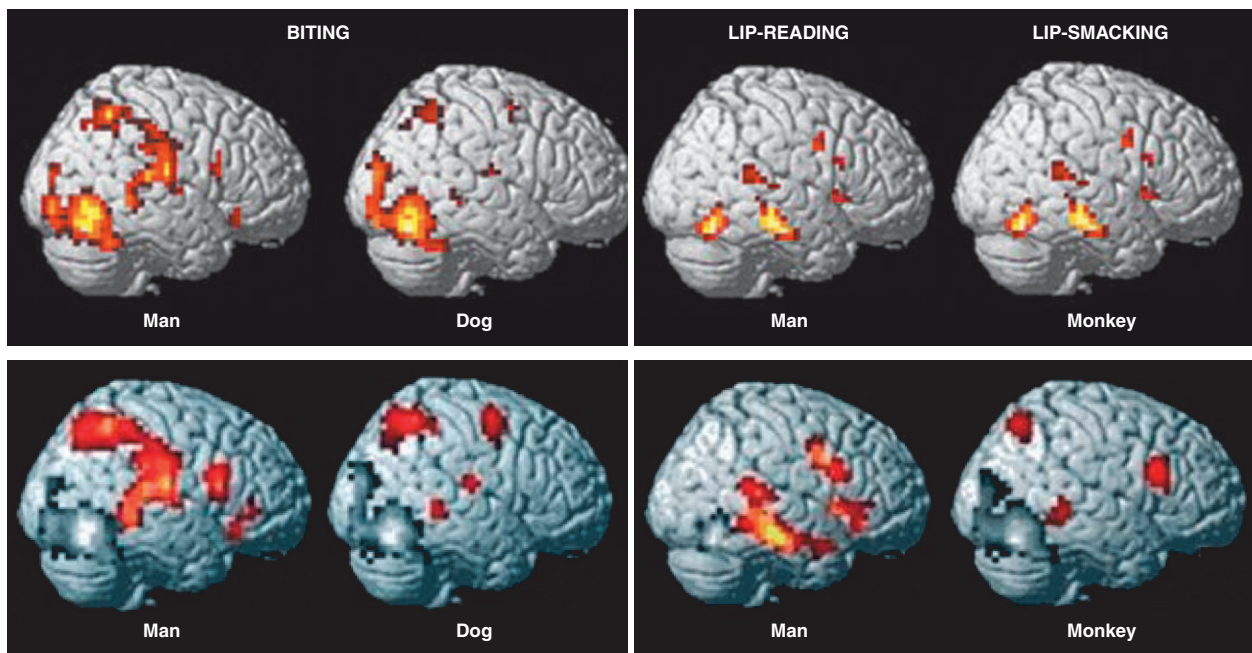
Figure 8. Buccino et al.'s (*Journal of Cognitive Neuroscience* 2004)[4] original figures 1 and 3 (*upper panel*) and figure 5.9 and 5.10 (*lower panel*) of Rizzolatti and Sinigaglia, "Mirrors in the brain", *Oxford University Press* 2008[47]. For brevity, only two representative sets are shown in this figure to highlight the differences between the two sets of "identical" images.

that the social deficits typical of these patients are down to a dysfunctional MNS, the experimental results so far produced in support of this are less than convincing when taken as a whole. Indeed, Figure 9 compares the results of an EMG experiment we carried out on non-autistic eight-year-old children with those reported by Cattaneo et al. in *Proceedings of the National Academy of Sciences of the USA* 2007[7], and later reiterated in figure 5 of Rizzolatti et al., *Nature Clinical Practice Neurology* 2009[44]. These Authors maintain that autistic children demonstrated no motor activation signalling anticipation of execution or mirroring during observation, unlike non-autistic children. When we repeated this experiment, however, on non-autistic children, the slope clearly shows that the EMG start rising after the child picked up the food (time zero) and it is far from the typically-developing child proposed in Rizzolatti et al., *Nature Clinical Practice Neurology* 2009[44], bearing a greater resemblance to the ASD slope.

Our experimental protocol and data processing were identical to those reported in Cattaneo's paper[7], assisted and overseen by a child neuropsychiatrist, a neurosurgeon and a neurophysiopathologist, and although further in-depth examination will be required, it is already evident that simple experimental protocols

and models cannot describe such complex systems. The discrepancy between the results of the two experiments (Cattaneo et al.[7], Pascolo et al.[38]) clearly demonstrates the need for careful interpretation of data before jumping to erroneous conclusions[24].

☐ DISCUSSION

Today most of the scientific literature takes the stance that the concept of mirror neurons is yet to be fully validated, particularly in humans. Indeed, MNS experiments carried out using fMRI possess enormous limitations associated with the purely haemodynamic method and probabilistic relevance[41], and this review, among others, has shown that even the very first invasive experiments on monkeys that led to the formulation of the paradigm itself should not be taken as gospel. What was in fact the object of measurement? Was it the "mirror" activity of a neuron, or simply an epiphenomenon involving a network of neurons? The departure point was at the very root of the research, the experiment documented in 1992[12]. Do U481 U483 neurons belong to a network? This appears to be the case, as they responded to the same stimulus, although in different forms and with different
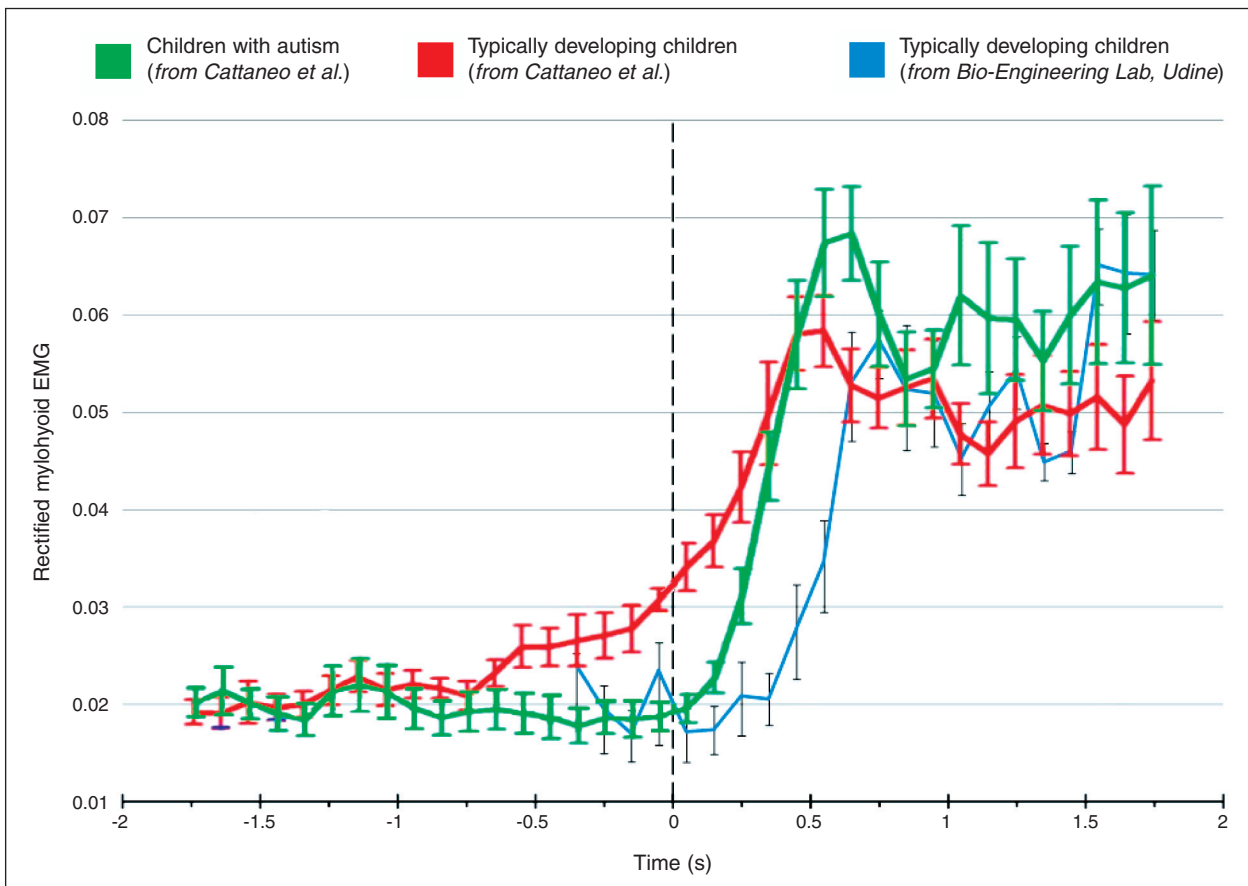
Figure 9. Comparison of the data proposed by Cattaneo et al., *Proceedings of the National Academy of Sciences of the USA* 2007[7] (*red and green lines*) with the experiment run at the Industrial Bioengineering Lab., University of Udine (*blue line*).

temporal modalities. However, this network aspect has not been studied in depth, even in the following works[20,45]. In fact, studies[12,20,45] have mainly focussed on the activities of single neurons in the cerebral premotor area F5 in response to elementary stimuli generated by a gratifying element, i.e., food. This narrow focus on single neurons, rather than the whole system, has no doubt played a part in generating the MNS paradigm.

In our opinion, however, in the seminal 1996 works[20,45], presented again in 2001 and 2006, not only did the monkey not mirror the experimenter, simple subtraction showed that it also probably anticipated him by 0.25 s[14]. In addition, the calculation of averages and the repositioning of recordings (histograms) give indications that are incompatible with the MNS paradigm. Indeed, it is possible to provide an example that demonstrates how results similar to those proposed in Figure 5 and Figure 7 of this work can be produced without the need to invoke the MNS.

Suppose that the reader of this paper is kitted out with dozens and dozens of hypothetical microelectrodes and just as many acquisition channels. A) First the hypothetical experimenter wonders aloud "How much is 6 multiplied by 6?" and then provides an answer "36." B) The experimenter then asks the reader "How much is 6 multiplied by 6?" and, the reader will undoubtedly say "36." It is likely that at least one neuron will fire during the first phase of the exercise (A), because "6 multiplied by 6?" is a well-known operation that brings to mind 36, and a second firing will be recorded when the reader will says the number 36 out loud (B): The result? A new Figure 5. Now suppose the experimenter asks him or herself "How much is 13 multiplied by 7?" and then responds "91". In this case, it is likely that weak activity during the neuron firing or no firing at all will be registered, whereas a clear signal will be detected when the reader is asked to respond to the question, thereby creating a new Figure 7. Is the MNS driving

the result or rather previous conditioning and motivation? Since there must be a strong correlation between brain activity and external (observable) activities, the logic and methods of the experiments used to demonstrate that this link is ascribable to the MNS theory leave much to be desired.

In 1988, Gentilucci et al.[23] state: "many F4 neurons responded passively to stimulation of the face and actively to arm movements [...] thus the organization of movement representation in F4 is different and more complex than that in F1". Although no mention was made of area F5, this experimental evidence confirms, if any such confirmation were needed, that the "electrochemical" information passing through the various neurons is based on various criteria (among which availability) and not on purely deterministic factors. The processes linked to execution are therefore more likely to be "locally" distributed rather than committed to certain neurons that carry out specific functions or exclusive activities. The neurons are all connected in different ways and by different paths. These connections can be understood in the light of the requirement to execute similar processes from time to time, but with neurons that may be different to those used previously. In the cases in question, one also has to consider the effect of the electrodes, placed near to or inside each other. Only a locally "decentralized" structure can survive an "attack" from the outside (electrodes, illnesses, etc.), as damage to a single neuron or a set of them (only few neurons were intercepted in the experiments) should not hinder the functioning of the whole brain system. i.e., redundant connections can be used to substitute the destroyed ones, and occupied neurons can be "replaced" by unoccupied ones.

Finally, if one considers that in the experiments examined, the neuron itself fired/did not fire according to the type of action observed, and fired during the execution, it follows that the neuron should not operate in the presence of concurrent and/or anticipated or unknown acts[46]. Hence, in functional and circuital terms the mirror effect is highly improbable.

Even if one examines the exercises presenting the neuron's delay with regard to the experimenters, MNS does not mimic the action observed with sufficient accuracy to make one think of a "copy" or simulation process (see numeric values reported in Figure 5 and 7). In other words, it is not simulation that allows the animal to understand what the meaning of an action is. The MNS paradigm would

therefore assume validity only at the end of the interpretation of the observed action (goal-oriented) and not at the beginning of the action itself[8]. The prevailing impression is that of an over-eagerness to construct a mind theory, even from the earliest experiments. Indeed, in di Pellegrino 1992[12] there is already a mention of the motor theory of perception, and the Authors hypothesize "... that premotor neurons can retrieve movements [...] also on the basis of the meaning of the observed actions".

The expression "mirror neuron", suggesting that there is a distinct population of neurons that perform a mirroring function, does not allow for the idea that common neurons, owing to more or less complex efficiency, also perform "mirroring" activities[51]. In other words, if there is a group of neurons that activate when a "grasping" gesture is executed, prepared or observed, this is just one of the many events that needs to take place in a network, or a subset of networks, required to manage a phenomenon as complex as that which controls motor action. While this control undoubtedly involves the prefrontal cortex as the prime agent, this chain of action will also involve the primary motor areas, the sensitive areas, the supplementary motor area, basal ganglions, cerebellum and the motor neurons of the spinal cord.

Understanding an object, action or circumstance must take place very quickly, and these recognition processes only need milliseconds via the visual path[26], for example. However, the firing characteristics of mirror neurons measured in the experiments are in the order of seconds, indicating that these are more likely to be an epiphenomenon of the recognition, approval and subsequent action planning or, even more simply, could pertain to prediction or anticipation of the action yet to follow, or all of these events put together: actual or virtual.

☐ CONCLUSION

In other words, the experiments analysed in this paper appear to measure the function undertaken by a group of neurons in a certain timeframe, rather than a property of a particular type of neurons that sets them apart. It is therefore expected that in the future we will recognize mirror-type activity in more or less coherent clusters of common neurons, thereby completely overturning the paradigm itself. It is this Author's belief that the doubts raised in this essay should be put to scientific debate for a more profound

reflection, as the falsification elements (Popper) of the paradigm are consistent and well correlated[10,25,35]. This is a particularly urgent matter, as despite the fact that the original paradigm has still to be fully accepted or rejected, it has already started to influence treatment protocols, particularly in the fields of rehabilitation and autism.

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