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# DO MACAQUES COUNT FROM LEFT TO RIGHT?

# NUMBER-SPACE ASSOCIATION AND THE ROLE OF EMOTIONS IN MACAQUES

Coordinatore:

Chiar.mo Prof. Luca Bonini

Tutori:

Chiar.mo Prof. Pier Francesco Ferrari

Chiar.mo Prof. Giorgio Vallortigara

Dottoranda: Giulia Annicchiarico

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# General Introduction

Numbers are a significant aspect of daily life, shaping our activities from the moment we check the time on our phones in the morning to routine tasks such as measuring ingredients while cooking or estimating distances to reach a specific destination. However, contrary to common belief, numbers also play a crucial role in the survival of several non-human species, ranging from mammals to arthropods. Studies of animals in their natural habitats suggest that numerical quantity representation influences daily decisions, such as during feeding. For instance, non-human animals use their ability to recognize and compare numerical magnitudes to select larger amounts of food, which directly impacts their foraging choices and enhances their chances of survival. Consequently, the study of numerical competencies from both behavioral and neurobiological perspectives in animals has become an increasingly interesting and developing research field.

This thesis aims to explore one of the most fascinating and contentious topics in numerical cognition: the connection between the representation of numerical magnitudes and spatial information in non-human species. This research aims also to investigate the origin and mechanisms underlying this association.

In the first chapter I will review scientific discoveries regarding the numerical perception and competence of non-human species, laying groundwork for further investigating how numbers are represented in space.

In the second chapter I will focus specifically on the number-space association in both human and non-human animals, highlighting the current debate about the biological or/and cultural origin of the phenomenon.

In the third chapter, I will present a series of studies conducted on *Macaca mulatta* and *M. fascicularis* aimed at expanding our understanding of the origins of the Number-Space Association (NSA). The first study aims to assess the presence of NSA in these two species, while the second aims to investigate the underlying mechanism of the phenomenon.

# Chapter I. *The "sense of number" in the animal kingdom*

Numerical competence, the ability to process and estimate numerosity, is no longer considered exclusive to humans, as previously believed until recent decades. Indeed, numerous studies have underscored its prevalence across the animal kingdom, from primates to insects (Brannon & Terrace, 1998; Gazzola et al., 2018; Howard et al., 2018; Potrich et al., 2015; Scarf et al., 2011; Uller et al., 2003).

These new findings suggest that numerical competence could represents an ancient mechanism conserved across species through evolution, and therefore, is likely not solely dependent on language.

The presence of a "sense of number", i.e. "the direct intuition of what numbers mean" (Dantzig, 1930; Deahene, 2011) among phylogenetically distant animal species supports the notion that numerical competence has likely evolved and persisted throughout evolution due to its adaptive value in nature (for a review see Neider, 2020).

Non-human species can derive significant benefits from the ability to estimate numerosity, i.e. the number of elements in a set, for essential survival behaviors such as foraging. For instance, when presented with multiple food options, some non-human animals can enhance their energy intake by selecting the more abundant food source (optimal foraging theory, Krebs et al., 1974).

The phenomenon of "going for more" has been demonstrated in various species of vertebrates (Abramson, 2011, 2013; Baker et al., 2011; Beran, 2001; Bogale et al., 2014; Garland et al., 2012; Gazzola et al., 2018; Hauser et al., 2000; Hunt et al., 2008; Lewis et al., 2005; Lucon-Xiccato et al., 2015; Stancher et al., 2015; Uller et al., 2003), as well as in invertebrates such as arthropods (Rodríguez et al., 2015) and mollusks (Yang & Chiao, 2016).

In cuttlefish (*Sepia pharaonis*), it has been demonstrated that they can discriminate between different quantities of shrimp, typically opting to feed on the larger group. However, they can also adjust their choice based on their hunger state and the quality of the prey (Yang & Chiao, 2016).

Estimating numerosity is also essential for other critical survival behaviors, such as locating a food source or navigating back to a familiar environment. For instance, honeybees (*Apis mellifera*) can count landmarks to assess travel distances (Chittka & Geiger, 1995), recall precise locations of food sources (Dacke & Srinivasan, 2008) and evaluate flower quantities within patches to optimize feeding efficiency (Howard et al., 2020).

Some non-human species are capable not only of estimating numerosity but also of demonstrating ordinal abilities, specifically the capacity to represent ordinal relations, such as identifying a target item in a series based on its ordinal position (Petrazzini et al., 2015; Potrich et al., 2019; Suzuki & Kobayashi, 2000). For instance, rats (*Rattus norvegicus*), when trained to choose the fourth box within an array, successfully identified it even when the boxes were rearranged or new ones were added (Suzuki & Kobayashi, 2000). Similarly, honeybees can use the number of landmarks as reference points to locate a food source (Chittka & Geiger, 1995), even when the distance to the reward is modified (Dacke & Srinivasan, 2008). Chicks (*Gallus gallus*), too, were trained to identify specific positions (the 3rd, 4th, or 6th) within a sequence of 10 equivalent locations, demonstrating accurate performance even when the array of stimuli was rotated by 90° and the spatial distances between positions were modified (Rugani et al., 2007). Using a similar protocol, Clark's nutcracker (*Nucifraga columbiana)* also demonstrated the ability to identify an object in a sequence of identical items based on its ordinal position (Rugani et al., 2010). Finally, Rugani and Regolin (2020) tested domestic chicks under monocular and binocular vision to investigate the role of each hemisphere in processing spatial versus ordinal-numerical cues. They found that while both hemispheres can independently process spatial and ordinal-numerical information, interhemispheric cooperation enhances the processing of ordinal-numerical cues, resulting in more efficient use of such knowledge (Rugani & Regolin, 2020).

Numerical competence also plays a crucial role in predator avoidance among non-human animals. In this context, preys employ various strategies, such as opting to join larger groups of individuals over smaller ones (Hager & Helfman, 1991). This strategy offers several advantages: larger groups reduce the risk of detection and predation for individual members, known as the "*dilution effect"* (Delm, 1990; Foster & Treherne, 1981). Additionally, larger groups increase the likelihood of predator detection, referred to as the "*many eyes effect*" (Roberts, 1996), or may cause confusion and disorientation in predators because of many similar-looking individuals, known as the "*confusion effect"* (Krakauer, 1995; Landeau & Terborgh, 1986).

However, some non-human animals employ an alternative strategy for avoiding predation by using alarm calls to alert other group members about the presence and level of danger posed by a threat. Numerical factors play a critical role in such cases. For example, in *Poecile atricapillus* (black-capped chickadees), the total number of notes per call provide information about the predator's size and the perceived level of threat (Templeton et al., 2005).

Another essential survival behavior involves defending one's territory, where estimating the number of individuals from an opposing group becomes crucial to assess the risk of harm or plan a defensive response. For instance, both lions (*Panthera leo*) and chimpanzees (*Pan troglodytes*) exhibit a reduced tendency to respond aggressively when confronted by a larger number of intruders compared to encounters involving fewer intruders (McComb et al., 1994; Wilson et al., 2001).

Lastly, the ability to assess numerosity also plays a significant role in reproductive strategies. In certain species, such as male tungara frogs (*Physalaemus pustulosus*), individuals may enhance their advertisement calls to outcompete rivals. Females demonstrate a preference for more complex calls, which are indicative of the males' vigor (Ryan, 1983)

Another intriguing behavior related to reproductive strategies influenced by quantity has been observed in males of pseudo-scorpions (*Cordylochernes scorpioides*). These arachnids possess the ability to regulate the amount of their ejaculate based on the number of competitor males that have previously copulated with the same partner (Bonilla et al., 2011).

Despite the widespread distribution of numerical competence across the animal kingdom, as discussed, it remains unclear whether this trait is homologous, inherited from a common ancestor and transmitted across diverse species, or if it has evolved convergently through independent evolutionary processes. In the latter case, numerical competence would have emerged independently multiple times across various categories of vertebrates and invertebrates (Ferrigno and Cantlon, 2017).

## *Representation of numbers: symbolic versus non symbolic*

Numbers can be represented in two distinct formats: symbolically, using Arabic numerals, and non-symbolically, through arrays of dots or other visual elements. Non-human animals (Vallortigara, 2012) and preschool children (Cordes & Brannon, 2009; Izard et al., 2009) typically rely on non-symbolic representations of numerosity up to the age of 4, prior to formal education, extracting numerical information from visual arrays of elements (Rugani et al., 2017).

It has been suggested that the primordial representation of numbers is non-symbolic, and that humans subsequently developed the symbolic representation based on this foundation (Dantzig, 1931; Deahene, 2011; Gallistel & Gelman, 2000; Neider, 2019). According to this perspective, when humans process Arabic numerals, they are mapped onto their related pre-encoded non-symbolic numerosities (Cantlon, et al., 2009; Dehaene & Cohen, 1995; Feigenson et al., 2004; Kutter et al., 2018; Nieder, 2016; Piazza, 2010; Piazza et al., 2007). Therefore, both representations rely on the same common magnitude system—the Approximate Number System (ANS) (Nieder, 2016; Piazza et al., 2007).

In contrast, other researchers support the idea that there are indeed two distinct systems: one for symbolic representation, wherein numbers are processed precisely, and another for non-symbolic representation, which allows for an approximate processing of numerosity (Krajcsi et al., 2016; Núñez, 2017; Reynvoet & Sasanguie, 2016). This perspective, which posits the existence of two separate systems, is supported by various behavioral observations (Marinova et al., 2018; Sasanguie et al. 2017).

Lyons and collegues for example gave support to this latter hypothesis by conducting a study in which human subjects were asked to compare two visual stimuli. These stimuli could either be both symbolic (such as words and digits), non-symbolic (a set of dots), or a combination of both (dots and Arabic numerals) (Lyons et al., 2012). The authors argued that if the underlying system for the two numerical representations were the same, performance should have been consistent across all three conditions. However, results indicated that participants were slower to respond in the 'mixed' condition. The authors proposed that this delay was attributable to the cognitive effort exerted by participants to shift from a symbolic to a non-symbolic system. This finding thereby supports the hypothesis of the existence of two distinct systems for numerical representation.

It is not clear yet whether there are two or a single system for symbolic and non-symbolic numerical representation. However, in this context, we will solely concentrate on non-symbolic representation, which is relevant to non-human animal species.

### *Two different systems for representing non-symbolic numbers*

The potential existence of two distinct systems concerns not only the symbolic versus non-symbolic representation of numbers but also numerical magnitude (small and large numbers). Based on different performance patterns towards small and large numerosities, it has been proposed that both humans and non-human animals possess two separate mental systems to represent non-symbolic numbers: the Object Tracking System (OTS) (Lassaline & Logan, 1993; Palmeri, 1997) and the Approximate Number System (ANS) (Dehaene, 2007; Nieder & Dehaene, 2009).

The ANS facilitates the estimation of an unlimited range of set sizes but in an approximate manner. It exhibits two key behavioral features: the numerical distance effect and the size effect. The numerical distance effect refers to the phenomenon where numbers that are farther apart (e.g., 3 and 6) are easier to distinguish than numbers that are close together (e.g., 3 and 4). Additionally, the size effect indicates that for a given numerical distance, larger numbers are consistently more difficult to discriminate than smaller ones. For example, distinguishing between 1 and 2 is easier than distinguishing between 11 and 12, even though the numerical difference between both pairs is the same (=1). To discern numbers precisely, such as 1 versus 2, the numerical distance must grow in proportion to the size of the numbers. Fundamentally, the capacity to distinguish between quantities is dependent on the ratio, meaning it fluctuates based on the proportional relationship between them.

The distance and size effects can be encapsulated by Weber's law ($\Delta I/I = c$) (Dantzig, 1930; Dehaene, 2011), which originates from the work of the physicist Ernst Heinrich Weber. Weber initially discovered this relationship in the mid-1800s, not in relation to numbers, but while studying the discrimination of object weights. He noted that humans perceive the relative difference in weight between two objects, focusing on the proportional difference rather than the absolute value (Weber, 1850).

Weber theorized that the ability to perceive differences between weights is proportional to the object's weight. For example, in order to distinguish between an object weighing 100 grams and another, a difference of at least 10 grams is needed, while a 200-gram object requires a 20-gram difference. This relationship is expressed by Weber's law ($\Delta I/I = c$), where $\Delta I$ is the noticeable difference and I is the reference weight. For weights, the constant c is 0.1. Indeed, substituting the values into the formula: 10 grams ($\Delta I$) / 100 grams (I) = 0.1 and 20 grams / 200 grams = 0.1. Weber's law applies not only to weight discrimination but also to numerical discrimination. The value of the Weber fraction varies among species, with lower values signifying superior discrimination abilities and higher values indicating inferior discrimination abilities, reflecting a reduced capacity to distinguish minor numerical differences (see Neider, 2019).

From an evolutionary perspective, the size and distance effects are adaptive, for example, in foraging contexts. In nature, it is more advantageous to distinguish between significantly different numbers of items (distance effect), such as two versus ten pieces of food, rather than small differences, like two versus three. Moreover, the ability to more easily discriminate small numbers also confers an advantage (size effect): for instance, distinguishing between one and two items is highly advantageous because two is double one, whereas distinguishing between 10 and 11 is often negligible.

In contrast, the OTS enables the precise, rapid estimation of small sets of objects (Pylyshyn, 2001; Treisman, 1992). Unlike ANS, the OTS has a "set-size limit" typically ranging from one to three or four items, though it can extend up to seven or eight items if they are familiar (Lassaline & Logan, 1993; Palmeri, 1997; Wolters et al., 1987). Thus, the OTS is specialized for discriminating only small sets of objects.

The key distinction between the OTS and the ANS lies in their mechanisms for perceiving numerosity. The OTS involves a highly precise perception of numerosity that relies on the absolute values of numbers, while the ANS operates primarily on the basis of their numerical ratios. Behavioral evidence for this distinction is supported by human studies indicating that for quantities up to four items, reaction times remain nearly stable, with an increase of approximately 40–100 milliseconds per item. In contrast, for larger numbers, reaction times increase significantly, by about 250–350 milliseconds per item (Atkinson et al., 1976; Fernberger, 1921; Jevons, 1871; Kaufman & Lord, 1949; Mandler & Shebo, 1982; Oberly, 1924).

However, it remains debated whether this system constitutes a distinct mental system and, if so, whether it is widespread among non-human species, with few studies showing evidence of OTS in animals (Agrillo et al., 2008; Hauser et al., 2000; Hunt et al., 2008). Many researchers argue that quantities ranging from 1 to 4 are not ratio-dependent and do not adhere to Weber's Law because they are small and easily discernible. Consequently, their perception may be governed by a system separate from the Approximate Number System (Dehaene & Changeux, 1993; Gallistel & Gelman, 1992; Van Oeffelen et al., 1982; Vetter et al., 2008).

Finally, it has been hypothesized that non-symbolic numerical cognition is integrated within a broader cognitive system that encompasses various types of magnitude representations, including space and time. This link raises the possibility that non-human animals may derive numerical information not solely from numerosity, but also from variables that co-vary with it, such as area, perimeter, and density (Leibovich et al., 2017).

For this reason, many studies on numerical discrimination in non-human animals have sought to control for these confounding variables, although this task is not always straightforward (MaBoudi et al., 2021). However, this does not invalidate the possibility that non-human animals use numerosity to estimate numerical quantities. Indeed, numerous studies have successfully shown that animals can rely exclusively on numerical cues (Nieder, 2019). For example, it has been demonstrated that rhesus macaques (*Macaca mulatta*), in a numerical match-to-sample task, base their decisions on numerical values rather than on shape, color, or surface area (Cantlon & Brennon, 2007b).

## *Mechanisms underlying numerical competence in non-human animals*

Since numerical competence is widely distributed in the animal kingdom, several studies have aimed to elucidate the underlying neural mechanisms of this ability, finding some evidence for a common shared system for estimating numerical information in both humans and non-human animals (for a review see Neider, 2021).

**Humans**. In humans, early research focused on patients with numerical calculation deficits, such as acalculia. These studies revealed that areas involved in symbolic calculations (Cipolotti et al., 1991) and the estimation of non-symbolic quantities (Ashkenazi et al., 2008; Lemer et al., 2003) include the inferior parietal lobule (angular gyrus), the intraparietal sulcus (IPS), and the inferior frontal gyrus (Henschen, 1919).

Subsequently, with more advanced functional imaging techniques, researchers identified that areas implicated in numerical competence also included parts of the posterior parietal cortex (PPC), particularly the IPS, the superior parietal lobe, and the prefrontal cortex (PFC) (Arsalidou and Taylor, 2011; Harvey et al., 2013; Jacob and Nieder, 2009; Piazza et al., 2004), with the involvement of the superior, medial, and inferior frontal gyri, the precentral gyrus, the cingulate gyrus, and the left fusiform gyrus (Arsalidou and Taylor, 2011).

Furthermore, "*number neurons*" have been recently explored in humans (Kutter et al., 2018), after have been found in non-human primates (Nieder et al., 2002; Nieder & Miller, 2003; Nieder, 2016), corvids (Ditz & Nieder, 2015) and chicks (Kobylkov et al. 2022).

These neurons are so named because they selectively respond to a specific preferred numerosity. For example, when presented with a set of dots, a neuron selective for "number 3" activates in response to various numerosities but shows the highest firing rate for the numerosity "3," with firing rates gradually decreasing for more distant numerosities (Fig.1).

The coding characteristics of *number neurons* can elucidate both the distance effect and the size effect (Nieder, 2020). The distance effect, which refers to the increased difficulty in distinguishing numbers that are numerically close, is due to the significant overlap in the tuning curves of number neurons. This overlap results in less distinct neural responses for similar numerosities, making it harder to discriminate between them.

In contrast, the size effect, which describes the greater difficulty in discriminating larger numbers compared to smaller ones with the same ratio, is attributed to the broadening of neuronal tuning functions as the preferred numerosity increases. As the numerosity becomes larger, the tuning curves of number neurons become wider, leading to less precise differentiation between larger quantities.

While there is more robust evidence for *number neurons* in non-human animals, in humans they have only been identified in patients undergoing treatment for epilepsy with implanted electrodes in the medial temporal lobe (MTL) (Kutter et al., 2018). This highlights the need to investigate their presence in healthy individuals as well.

**Non-human primates**. Non-human primates were the first order of animals in which *number neurons* were explored and identified, with studies predominantly focusing on macaques. These investigations have revealed the presence of number neurons in the frontal, parietal, and temporal associative cortices (Nieder, 2012; Nieder, 2016; Nieder et al., 2002; Nieder et al., 2006; Nieder and Miller, 2003; Okuyama et al., 2015). Additionally, these neurons are not affected by physical changes in stimuli (Nieder et al., 2002), responding more to numerical information than to other non-numerical variables that may co-vary with the stimuli.

In the prefrontal cortex (PFC), some *number neurons* respond to identical numerical values regardless of whether events are visual or auditory, demonstrating their ability to respond to numerosity regardless of the modality of the stimuli (Nieder, 2012). *Number neurons* in non-human primates are predominantly located in the prefrontal cortex (PFC) and the posterior parietal cortex (PPC), mostly in area VIP (ventral intraparietal area) in the fundus of IPS (Jacob and Nieder 2014; Nieder et al., 2006; Nieder and Miller, 2004; Tudusciuc and Nieder 2009), with relatively few found in the inferior temporal cortex (Eiselt and Nieder, 2013; Nieder and Miller 2004; Vallentin et al., 2012).

**Birds and other species**. Neurons that responded mostly to specific number of stimuli have also been identified in cats (*Felis catus*), specifically in the posterior region of the middle suprasylvian gyrus (PMSA) though they are present in very low numbers (Thompson et al., 1970). However, further research is necessary, as this study constitutes the initial investigation into this phenomenon, and the identification of these neurons was incidental. More robust evidence of *number neurons* has, instead, been found in corvids (Ditz & Nieder, 2015) (Fig.2).

Indeed, crows (*Corvus sp.*) exhibit highly flexible behaviors (Emery and Clayton, 2004), and advanced numerical competence. For instance, they can discriminate between quantities and recognize empty sets as a numerical category (Kirschhock et al., 2021). *Number neurons* have been found in the caudolateral nidopallium (NCL), which is a high-level pallial integration center (Ditz and Neider, 2015; Moll and Neider, 2015; Nieder et al., 2020; Veit et al., 2015). Similar to those in primates, *number neurons* in corvids also exhibit a neuronal distance and size effect that conforms to Weber's law.

Recently, Kobylkov and colleagues (Kobylkov et al. 2022) identified *number neurons* in the caudal nidopallium (NCL) of young domestic chicks, a species already recognized for its numerical capabilities, including the ability to differentiate between quantities (Rugani et al., 2008; Rugani et al., 2013) and to perform basic arithmetic tasks (Rugani et al., 2009). The findings suggest that these *number neurons* exhibit characteristics similar to those identified in adult primates and crows, suggesting that the ability to perceive numerosity, grounded in *number neurons,* may be an inherent feature of the vertebrate brain.

The involvement of pallial regions in numerical competencies seems to be a common feature among vertebrates. In mammals, the dorsal part of the telencephalic pallium has evolved into the neocortex, where associative areas are responsible for « the number sense ». In other vertebrate groups, such as birds and fish, different regions of the pallium have evolved distinct structures but still appear to play a role in numerical processing.

**Other Vertebrates.** In fish, behavioral evidence has demonstrated numerical competence (Agrillo & Bisazza, 2018), with performance conforming to Weber's law (Agrillo et al., 2008). The regions involved in numerical processing include the thalamus and telencephalon (Messina et al., 2020). Notably, activation of the caudal portion of the dorso-central division of the pallium has been observed during number-related tasks (Messina et al., 2022). Recent discoveries (Luu et al., 2024) have identified neurons in larval zebrafish that specifically respond to numerical stimuli, highlighting the significant role of early numerical cognition for survival during the initial stages of life, even prior to the emergence of any recognized number-related behaviors (Adam et al., 2024; Borla et al., 2002; Lucon-Xiccato et al., 2023).

In amphibians, which respond to basic quantitative information (Krusche et al., 2010; Stancher et al., 2015; Uller et al., 2003), it remains unclear whether they can process more complex numerical stimuli. The torus semicircularis, located in the midbrain of frogs, appears to be involved in numerical processing of auditory stimuli (Alder & Rose, 1998). Neurons in this region are activated by consistent call sequences and show firing activity when these sequences include an additional pulse, suggesting an ability to "count" fixed inter-pulse intervals (Edwards et al., 2002, 2007; Naud et al., 2015; Rose, 2018). However, these neurons seem to function only with regular calls that have fixed intervals and do not react to sequences with variable/mixed intervals.

**Protostomes.** Concerning protostomes, which are characterized by a ventral nerve cord and a circumoesophageal anterior brain, as opposed to deuterostomes with a central dorsal nervous system, different hypotheses about the neural correlates of numerical abilities have been proposed.

Among insects, bees have been the most extensively studied for numerical abilities. They seem capable of developing abstract concepts related to absolute numerosity, demonstrating not only the ability to discriminate between large and small quantities but also to understand ordinal rank (Bortot et al., 2019; Howard et al., 2018). It has been suggested that in these species, an initial estimation of numerosity occurs in the optic lobes, where neurons automatically process visual numerosity (Paulk et al., 2014).

For more complex numerical abilities, it appears that the central complex, which is thought to be homologous to the vertebrate basal ganglia (Strausfeld & Hirt, 2013b), may play a role. In humans, the basal ganglia are similarly engaged during computational tasks (Arsalidou & Taylor, 2011).

In arthropods, the bilateral mushroom bodies located in the protocerebrum may serve as a key region for abstract and multimodal numerical representation. Their structure and functionality bear resemblance to the organization of certain mammalian regions, including the cerebellum and pallial areas such as the hippocampus, amygdala, and prefrontal cortex (Farris, 2011; Menzel, 2014; Owald and Waddell, 2015; Tomer et al., 2010). These mammalian structures are known to play a role in numerical processing in humans (Arsalidou and Taylor, 2011; Kutter et al., 2018).

Lastly, certain spiders, such as the spider-eating spider (*Portia africana*), have been observed using numerical information in their feeding behaviors. These arthropods assess whether to approach another spider's nest depending on the number of conspecifics located there (Nelson & Jackson, 2012). The protocerebrum, particularly the mushroom bodies, is thought to play a role in processing numerical information (Hartenstein, 2017; Long, 2021).

Furthermore, a recent study (Bengochea et al., 2023) has demonstrated that *Drosophila melanogaster*, commonly known as fruit flies, possess the ability to differentiate between quantities. These flies showed a preference for larger groups of objects, with their performance being influenced by the ratio of the two numerical quantities presented, suggesting a common feature with other species. The neuronal foundation for this numerical discrimination has been identified in lobula columnar neurons (LC11), indicating that these may serve as a neuronal basis for numerical processing in invertebrates.

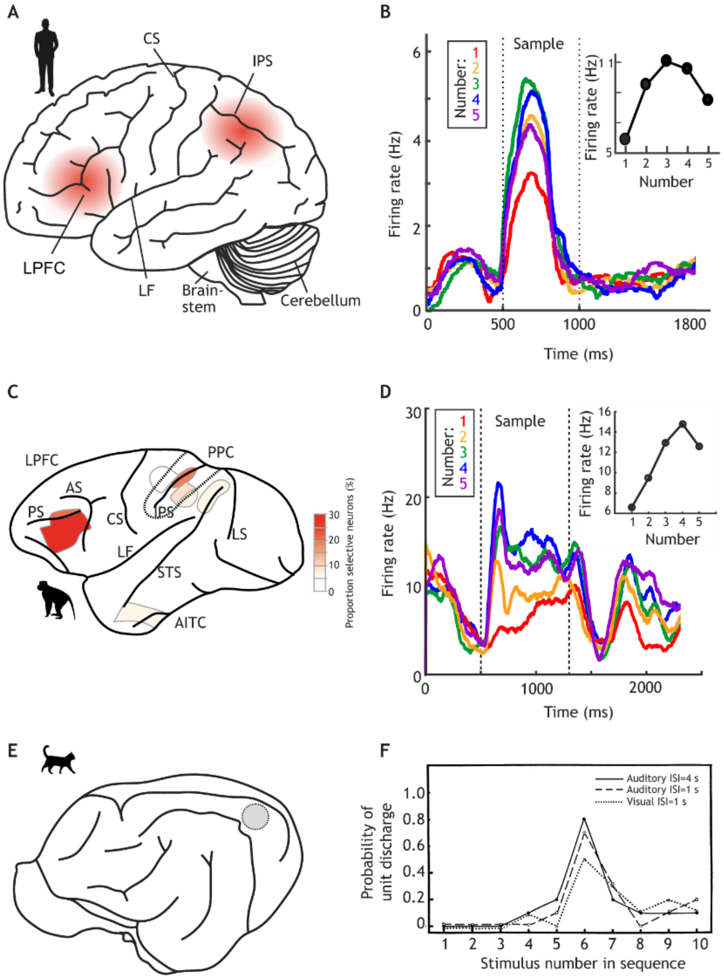


***Figure 1.*** *Representation of numbers in the brains of humans (A), rhesus monkeys (C), and cats (E). A neuron responsive to the number '3,' recorded from the medial temporal lobe (MTL) of human patients, exhibited the highest firing rate for the preferred numerosity of 3 (B); a neuron responsive to the number '4,' recorded from the ventral intraparietal (VIP) area of a rhesus monkey performing a numerical discrimination task (D); and a neuron responsive to the number '6,' recorded from the PMSA of an anesthetized cat (F). The figure and description are taken from Nieder, 2021.*

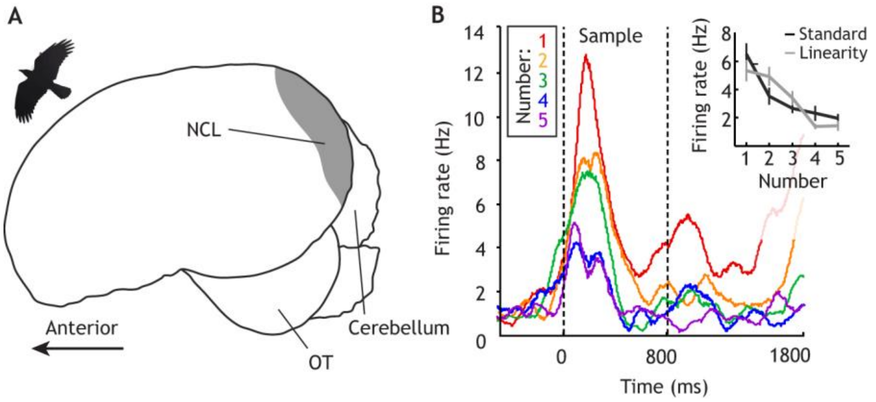


***Figure 2.*** *Number encoding in the brain of crows. (A) A side view of a crow's brain highlights the nidopallium caudolaterale (NCL, shaded area) located in the telencephalon, with the optic tectum labeled as OT. (B) An example of a neuron responsive to the number one was recorded from the NCL of crows while they performed tasks involving numerical discrimination, exhibiting its peak firing rate specifically for the quantity '1.' Figure and description adapted from Ditz and Nieder, 2015, and Nieder, 2021.*

## *Behavioural studies on numerical competence*

As discussed in the previous chapter, the neural correlates of an innate and intuitive sense of numerosity have been investigated across nearly all vertebrate classes, including species that are highly diverse and phylogenetically distant. To gain a more comprehensive understanding of numerical competence in the animal kingdom and to adopt a comparative approach, it is essential to investigate behavioral research on the 'sense of number' in non-human animals.

Behavioral studies on numerical abilities in animal species generally employ two primary approaches: the spontaneous choice test and the trained behavior test. The spontaneous choice test offers ecological validity and allows for quicker experimentation without requiring a training phase. However, this method has limitations, particularly in its capacity to evaluate more complex tasks.

In contrast, the trained behavior test involves a training phase conducted within a more controlled laboratory setting, enabling the assessment of more complex tasks, such as those using computer-based stimuli that can be precisely controlled for continuous variables. However, this approach typically lacks ecological validity. Both methods are valuable and have been employed to test different hypotheses, each contributing crucial insights into numerical capabilities and enriching our comprehensive understanding of this phenomenon.

In non-human primates, one of the pioneering studies on numerical competence was conducted by Hebert S. Terrace and Elizabeth M. Brannon in 1998 (Brannon & Terrace, 1998, 2000). The researchers trained two rhesus macaques to arrange sets of dots displayed on a touchscreen in ascending order.

To ensure that the monkeys relied solely on numerical cues, the researchers controlled for non-numerical variables such as size, area, and shape of the stimuli (Fig.3). The macaques rapidly learned to arrange numerosities from smallest (i.e., numerosity 1) to largest (i.e., numerosity 4), and they successfully performed the task with novel stimuli and numerosities up to nine, which they had not previously encountered. These results demonstrated that the macaques could generalize and apply the numerical ascending order rule to new and unfamiliar sets of stimuli.

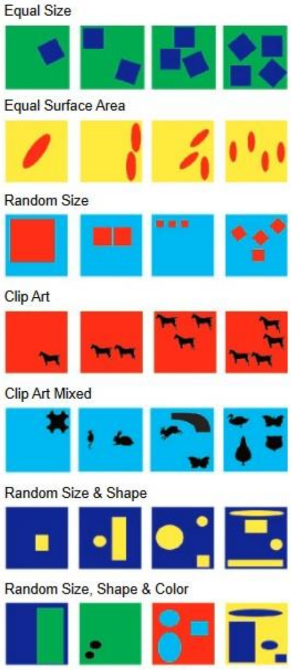


***Figure 3.*** *An example of the different types of stimuli used by Brannon & Terrace (1998). Stimuli with equal size and shape (Equal Size); stimuli with the same cumulative area (Equal Surface Area); stimuli with varying sizes (Random Size); non-geometric elements of the same size (Clip Art); non-geometric elements of various sizes (Clip Art Mixed); geometric elements varying in both size and shape (Random Size & Shape); and geometric elements varying in size, shape, and color (Random Size, Shape & Color).*

Monkeys also demonstrated proficiency in a delayed match-to-sample task, where a numerosity, presented as dots, was displayed on a monitor. After a delay, another numerosity was shown, either matching or differing from the initial one (Fig.4B). The monkeys were required to respond only if the numerosity matched that of the previous trial (Nieder et al., 2002). Furthermore, the monkeys' numerical abilities appeared to follow Weber's law, as their performance declined when the numerical distance between the non-matching and matching stimuli was minimal.

This study demonstrated that macaques, as has been shown also in chimpanzees (*Pan troglodytes*; Matsuzawa, 1985; Murofushi, 1997), pigeons (*Columba livia*; Hiray & Jitsumori 2009; Xia et al., 2001), parrots (*Psittacus erithacus*; Pepperberg, 1994) and corvids (*Corvus corone corone* and *Corvus cornix*; Ditz and Nieder, 2016; Smirnova et al., 2000), can flexibly distinguish one numerosity from another, indicating that they possess a representation of numbers based on their absolute value.

The representation of absolute numerosity is considered a more advanced cognitive skill in comparison to relative quantity judgment. Relative quantity judgment entails the ability to discern whether a quantity is greater or smaller than a reference numerosity. This form of numerical competence is considered more fundamental/basic, as it does not necessitate the representation of absolute values.

Another aspect of numerical competence involves sequential numerical presentation, where different numerosities are presented successively, requiring them to be "counted" and held in mind. Nieder and colleagues (2006) tested this ability in rhesus macaques (Nieder et al., 2006). In their task, numerosities were presented sequentially (Fig. 4A). For example, the numerosity 1 was displayed four times with brief pauses between each presentation, followed by a matching (4 dots) or non-matching stimulus (another quantity). The macaques successfully completed the task, demonstrating an understanding of sequential numerosity.

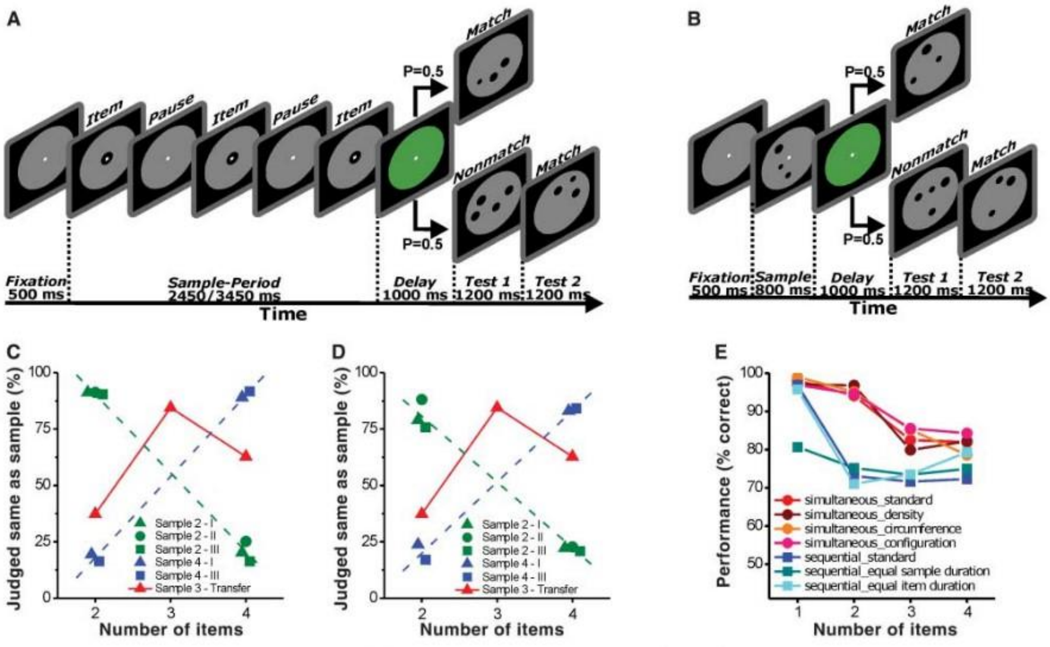


*Figure 4. An example of task protocols and behavioral performance from Nieder et al. (2002, 2006). Two delayed match-to-sample tasks: in the first experiment (A), the monkey had to match a sequential numerosity (e.g., three instances of "1") to its total quantity after a delay; in the second experiment (B), the monkey had to match an absolute numerosity (e.g., a single "3") to the same number after a delay.*

Jhordan and collegues (Jhordan et al., 2008) subsequently introduced an additional challenge to the task to investigate whether macaques could integrate different modes of numerical presentation, such as visual and auditory stimuli. In this extended version of the match-to-sample task, sounds were incorporated alongside visual stimuli. For instance, monkeys heard a sequence of five sounds (ranging from 1 to 9), followed by the presentation of five dots on the screen (match stimulus) or a different numerosity (non-match stimulus). The monkeys accurately matched the auditory numerosities with the visual ones, demonstrating their ability to integrate information across modalities. They performed this task as successfully as they did with single-modality stimuli (visual-visual), indicating a genuine understanding of numerical concepts.

Among non-human primates, not only macaques possess the ability to discriminate numerical quantities. This capability has also been observed in orangutans (*Pongo pygmaeus*; Anderson et al., 2007), gorillas (*Gorilla gorilla*; Anderson et al., 2005), chimpanzees (*Pan troglodytes*; Beran 2001; Beran & Beran, 2004), as well as squirrel monkeys (*Saimiri sciureus*; Thomas et al., 1980), baboons (*Papio hamadryas*; Smith et al., 2003), lemurs (*Eulemur mongoz*; Lewis et al., 2005) and capuchin monkeys (*Sapajus apella*; Beran et al., 2008; Judge et al., 2005).

For example, Addessi and colleagues (Addessi et al., 2008) subjected ten *Sapajus apella* to relative numerousness judgment tasks. The subjects were asked to choose the larger amount between two quantities of the same food and, in a subsequent experiment, between two quantities of tokens. In both cases, the monkeys successfully selected the larger quantity, indicating their ability to distinguish between different number of stimuli. Moreover, the monkeys performed better in the food-based tasks compared to those involving tokens. The researchers attributed this difference to (1) the significant cognitive demands associated with the token task, and (2) a reduction in motivation caused by the lag in feedback tied to token exchanges, indicating that food-based paradigms might be more effective for tasks requiring sustained motivation (Addessi et al., 2008)

Mammals have also been extensively studied for their numerical abilities, particularly through spontaneous choice tasks. Many species have demonstrated at least basic relative quantity judgments: dogs (*Canis lupus familiaris*; West & Young, 2002), cats (*Felis catus*; Pisa & Agrillo, 2009), bears (*Ursus americanus*; Vonk & Beran, 2012), lions (*Panthera leo*; McComb et al., 1994), hyenas (*Crocuta crocuta*; Benson-Amram et al., 2011), sea lions (*Otaria flavescens*; Abramson et al., 2011), raccoons (*Procyon lotor*; Davis, 1984), dolphins (*Tursiops truncates;* Jaakkola et al., 2005 ; Kilian et al., 2003), whales (*Delphinapterus leucas*; Abramson et al., 2013), horses (*Equus caballus*; Uller & Lewis, 2009), elephants (*Loxodonta africana;* Perdue et al., 2012), and rats (*Rattus norvegicus*; Davis & Albert, 1986; Fernandes & Church, 1982).

Moving beyond a phylogenetic perspective, there is substantial evidence of advanced numerical competencies in birds. Notably, crows exhibit sophisticated numerical skills and possess neural mechanisms akin to those found in macaques and humans, including "number neurons". Additionally, laboratory studies have been conducted on the African grey parrot (*Psittacus erithacus*; Pepperberg, 1987) and pigeons (*Columba livia*; Xia et al, 2001), further demonstrating the extent of avian numerical abilities.

These two species, like crows, exhibit flexible numerical competence with the ability to distinguish absolute numerosities and arrange them based on their ranking values (Scarf et al., 2011). Remarkably, even chicks exhibit significant numerical abilities. Researcher G. Vallortigara has referred to them as "natural born mathematicians" due to their capacity not only to discriminate quantities but also to perform basic addition and subtraction operations (Rugani et al., 2008).

In amphibians, intriguing evidence of numerical competence has been observed. For example, salamanders (*Plethodon cinereus* spp.) were seen choosing the jar with more fruit flies, distinguishing between quantities of up to 2 versus 3 (Uller et al., 2003). However, subsequent experiments did not confirm that these foraging strategies were based solely on numerosity rather than on other covarying variables (Krusche et al., 2010).

In contrast, *Bombina orientalis* has demonstrated the ability to rely exclusively on numerical cues, successfully discriminating between two quantities even when other variables were controlled (Stancher et al., 2015).

Surprisingly, reptiles have been largely understudied. In the lizard *Podarcis sicula*, several studies have failed to find evidence of quantity discrimination in this species (Petrazzini et al., 2017; 2018). However, recent research has demonstrated that Hermann's tortoises (*Testudo hermanni*) are capable of selecting the larger food item when presented with two options (Gazzola et al., 2018), thereby indicating that reptiles are not an exception in the realm of numerical abilities. Conversely, there is more evidence for numerical competence in the class of fish.

Studies have been conducted on mosquitofish (*Gambusia holbrooki*), guppies (*Poecilia reticulata*), angelfish (*Pterophyllum scalare*), swordtails (*Xiphophorus hellerii*) and zebrafish (*Danio rerio*). These fish were tested using both spontaneous choice tasks and and training-based tests. For instance, in an experiment by Agrillo and collegues, researchers placed a fish in a tank with two doors: one displayed three shapes and the other four. Only the door with three shapes provided access to the partners. After training, the fish successfully chose the door with three shapes, demonstrating the ability to discriminate quantities (Agrillo et al., 2009) (Fig.5).

Zebrafish have also demonstrated numerical discrimination abilities. In a 2015 study by Potrich and colleagues (Potrich et al., 2015), male zebrafish preferred locations previously occupied by larger groups of females over those with fewer females, with their performance influenced by the ratio between the group sizes. Subsequent research confirmed that this ability to discriminate was based on numerical cues rather than other factors, such as spatial frequency (Adam et al., 2024).

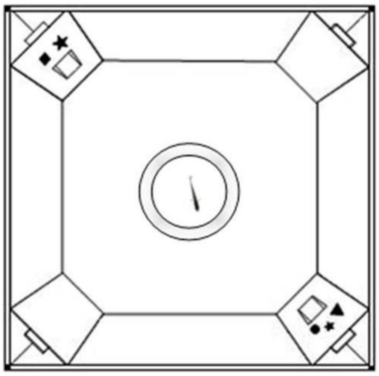


***Figure 5.*** *Apparatus used to train a fish, as described by Agrillo et al. (2009). The fish was trained to discriminate between two panels: one displaying three figures and the other displaying four figures, in order to join its mates.*

Lastly, recent studies in arthropods have revealed numerical competence in various species of spiders and insects, including beetles (*Tenebrio molitor*; Carazo et al., 2009), ants (*Formica polyctena* and *Cataglyphis sp.;* Reznikova & Ryabko, 1996; Wittlinger et al., 2006), and bees (*Apis mellifera* and *Bombus terrestris audax*; Bortot et al., 2019; Gross et al., 2009; Howard et al., 2020; MaBouDi et al., 2020). For example, honeybees have demonstrated their numerical abilities by performing match-to-sample tasks, where they remember and select previously shown numerosity (Gross et al., 2009), or by counting landmarks in a foraging gallery to obtain a food reward (Dacke & Srinivasan, 2008).

These findings underscore the widespread presence of numerical abilities across the animal kingdom, with documented examples in nearly all vertebrate classes. Both human (Moyer e Landaeur, 1967) and non-human animals (Cantlon e Brannon 2007a; Scarf et al., 2011) exhibit improved performance in non-symbolic numerical tasks when the distance between numbers is greater (distance effect) and decreased performance as numerical magnitude increases (size effect). This suggests a common, ancient non-verbal numerical mechanism (Cantlon e Brannon, 2006).

# Chapter II. *Number Space-Association*

In recent years, research on numerical skills has expanded beyond numerical discrimination abilities to investigate how numbers are associated with space, a phenomenon known as "Number Space-Association." Substantial evidence indicates that, in left-to-right reading cultures, humans conceptualize numbers along a continuous mental line with a horizontal orientation from left to right, referred to as the Mental Number Line (MNL). For instance, when considering a numerical range from one to ten, individuals typically organize these numbers mentally from left to right.

The first evidence that individuals mentally visualize numbers in spatial forms was provided by Francis Galton, who published "Visualised numerals" in 1880 and "The Visions of Sane Persons" in 1881. In these pioneering studies, Galton documented the drawings created by participants, revealing that most individuals experience numbers as vivid visual forms, called by the author "number-form" (NF), with distinct spatial arrangements. Notably, he observed that the configuration of NF varied among participants: while some described it as horizontally oriented, others reported vertical or mixed orientations (Fig. 6).

These findings were subsequently corroborated by three studies conducted by Jacques Bertillon (Bertillon, 1880, 1881), who extended the observation to include ordinal information such as months of the year and days of the week (Fig.7). Bertillon noted that such ordinal information was also spontaneously organized into spatial configurations. A century later, Seron and colleagues (1992) examined this topic and found that while the structure of Number Forms (NF) can vary greatly between individuals —manifesting as patterns as lines, grids, or shifts in position/color of the initial number—the spatial configuration of the NF was highly stable for each individual. Each number retained a fixed position and consistent shape within an individual's spatial framework. Moreover, participants reported that merely perceiving a number— whether via auditory, visual, or mental representation —could automatically activate these spatial frameworks.

However, the first substantial evidence of the mental number line and its behavioral effect emerged one year later, in 1993. Dehaene and colleagues conducted a study where participants were asked to judge numbers ranging from 0 to 9, which appeared at the center of a monitor, as either even or odd. During the task, participants were instructed to press the left button if the number was odd and the right button if it was even, with the rule reversed in a subsequent phase. The study revealed that participants responded more quickly with the left hand for smaller numbers (closer to zero) and with the right hand for larger numbers (closer to nine). This phenomenon was termed the SNARC effect, or Spatial Numerical Association of Response Codes (Dehaene et al., 1993).

To determine whether the observed spatial arrangement of numbers was due to side-biased spatial organization or hand-biased response tendencies, the authors replicated the experiment with participants' hands crossed. In this condition, participants still judged smaller numbers more quickly by pressing the left button, even when using their right hand, and larger numbers more quickly by pressing the right button, even when using their left hand. Another significant issue raised by the authors was whether the mental number line was influenced by the left-to-right direction of reading and writing numbers in Western cultures, given that all the participants were French. To address this, the authors replicated the task with individuals of Iranian origin who had moved to France. They found no evidence of an SNARC effect or a reversed effect among these participants. However, they observed that those who had lived in

France for a longer period displayed performance patterns more similar to the French participants.

Subsequently, several studies aimed to explore whether cultural factors influenced the orientation of the mental number line. For instance, Shaki and colleagues (2009) replicated the task used by Dehaene et al. (1993) with participants from different writing cultures. They tested Canadians, who write both words and numbers from left to right; Palestinians, who write both numbers and letters from right to left; and Israelis, who write letters from right to left but numbers from left to right. The results of the study revealed that Canadians exhibited a left-to-right spatial association with numbers, while Palestinians demonstrated a reverse association, from right to left. In contrast, Israelis showed no significant spatial association of numbers. The authors interpreted this latter finding as a consequence of competing influences between the two directions of writing—numbers from left to right and letters from right to left—affecting the spatial representation of numbers in Israelis.

Recently, a study conducted by Pitt and colleagues in 2021 tested both adult Tsimane from an indigenous Bolivian culture characterized by an oral tradition and preschoolers from the United States. The task required participants to arrange cards based on the numerical value represented by dots, ranging from 1 to 5. The study found no evidence supporting the presence of a SNARC effect in either group (Pitt et al., 2021). Although these associations are automatic, this study, along with others (Shaki et al., 2009; Shaki & Fischer, 2008; Zebian, 2005), has sparked debate over whether the mental number line is rooted in biological factors or shaped exclusively by cultural influences. Most findings support the notion that the MNL is a trait emerging from cultural context rather than a cognitive factor present independent of education, while others suggest that both cultural and cognitive components coexist in the development of this phenomenon (Eccher et al., 2023)

Despite the origins of the SNARC effect remaining uncertain, further evidence has shed light on various aspects that characterize it. For instance, studies have shown that the SNARC effect is not due to hand bias but rather reflects a genuine spatial side-bias. This has been demonstrated by observing the phenomenon not only in manual responses but also in tasks where individuals make parity judgments by pointing (Fischer, 2003) or by executing saccades (Schwarz & Keus, 2004).

Furthermore, the SNARC effect has been extended to other spatial dimensions. Research suggests that smaller numerical values are associated with downward spatial directions, while larger values are linked with upward directions (Hartmann et al., 2014). For example, Japanese participants exhibit faster response times to smaller numbers when using a button located lower and to larger numbers when using a button positioned higher (Ito & Hatta, 2004), even though Japanese writing can be arranged either horizontally from left to right (similar to Western cultures) or vertically from top to bottom.

The existence of a 'vertical' mental number line is further supported by evidence showing that larger numbers improve the perception of stimuli in the upper part of the visual space, while smaller numbers enhance the recognition in the lower portion of the visual field (Pecher et al., 2010)

Cultural factors have been proposed as a potential origin for this vertical association, such as the arrangement of numbers on elevators or thermometers (Hubbard et al., 2005). However, it remains debated whether the horizontal or vertical mental number line is more prevalent (Gertner et al., 2013; Wiemers et al., 2014; Winter et al., 2015).

Furthermore, the SNARC effect may vary depending on the context. For instance, Bächtold and colleagues demonstrated that adults exhibit a typical SNARC effect when conceptualizing numbers as distances on a ruler. Conversely, when numbers are conceptualized as hours on a clock face, the SNARC effect is reversed (Bächtold et al., 1998). Additionally, engaging in a brief session of finger counting in the reverse direction can potentially influence the direction of the SNARC effect (Pitt & Casasanto, 2014).

Lastly, the SNARC effect is thought to extend beyond numbers to other ordered sequences, such as letters, days of the week and months of the year (Gevers & Reynvoet, 2003; 2004). It also influences spatial dimensions, like the size of a grid aperture. For example, a study found that smaller numbers prompted quicker hand closure responses, while larger numbers elicited quicker hand opening responses (Andres et al., 2004). Collectively, these findings suggest that numerical-spatial interactions are not confined to specific effectors but occur within stable, effector-independent spatial-coordinate frames.

Despite the extensive research on the SNARC effect, several aspects remain still unresolved, particularly regarding the origins of the Mental Number Line (MNL) and the relative contributions of cultural versus biological factors. To explore these issues, recent studies have examined the occurrence of the SNARC effect in pre-verbal infants and non-human animals - not affected by cultural influences. These investigations (Bulf et al., 2015; de Hevia et al., 2014a-b; Di Giorgio et al., 2019; Giurfa et al., 2002; Rugani et al., 2015b; Rugani et al., 2024) have offered evidence supporting the notion of a universal, biological, and potentially innate association between numbers and spatial representation.

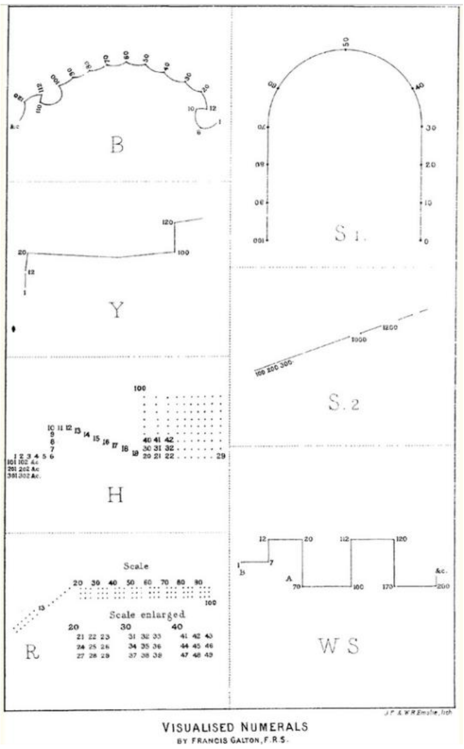


*Figure 6. Francis Galton was the pioneer in documenting externalized "number forms" or representations of numbers in spatial configurations. These visualizations of how numbers are spatially distributed were illustrated by six synesthetes and are depicted in plate VII of the appendix to Galton's 1881 publication.*

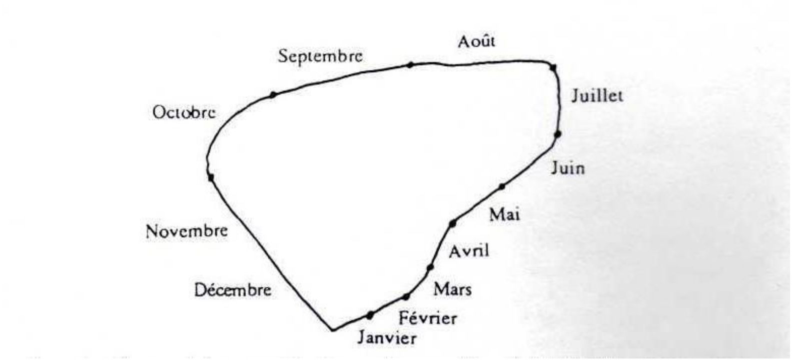


***Figure 7.*** *Visuo-spatial representations for months as reported by Bertillon (1882)*

## *Number Space-Association in pre-verbal infants*

Given the difficulties in isolating cultural and linguistic influences on the orientation of the mental number line (MNL) in adults, recent research has focused on preverbal infants. These studies, which predominantly use looking-time measurements to investigate an attentional SNARC effect, suggest that culture is not the primary determinant of the MNL. Rather, while cultural factors may influence the orientation of the mental number line, they are not the fundamental cause of its existence (for a review see Rugani & de Hevia, 2017).

In 2010, de Hevia and Spelke investigated the ability of 8-month-old infants to link numerical and spatial information. The study began with a habituation phase where half of the infants were exposed to ascending numerical sequences (array of visual elements, e.g., dots, in ascending order), while the other half to descending sequences. After habituation, the researchers assessed whether infants could transfer the concept of numerical order to spatial information by using lines of varying lengths. The results revealed that infants who were habituated to ascending numerical sequences looked longer at lines that decreased in length, and those habituated to descending sequences looked longer at lines that increased in length (de Hevia and Spelke, 2010). This suggests that infants were able to link numerical information with spatial patterns.

In a subsequent experiment the researchers combined numerical and spatial information by presenting infants with images where longer lines corresponded to more dots (positive relationship). Infants who had been habituated to this congruent pairing (long lines with many dots) showed a preference for new images that maintained the same pattern. Conversely, infants who had not been habituated exhibited a general preference for congruent pairings over incongruent ones (Fig. 8).

These findings suggest that infants are capable of linking numerical and spatial information and show a bias for consistent, congruent patterns. Moreover, they indicate that the association between numerosity and spatial information, such as length, arises during the early stages of life, even before infants receive formal education. To explore whether this phenomenon is *amodal*— that is, not restricted to a single modality but potentially emerging from multiple modalities—the same authors conducted further experiments using a multimodal approach, incorporating both auditory and visual inputs.

They habituated newborns (less than 96 hours old) to sequences of sounds (sequences of syllables) paired with lines of varying lengths: a congruent condition with few sounds and short lines or many sounds and long lines, and an incongruent condition with mismatched pairs. After habituation, the infants were shown two new stimuli—one with a congruent pattern and one with an incongruent pattern. The newborns consistently looked longer at the congruent pattern, regardless of their habituation condition. These results indicate that even newborns are sensitive to congruent patterns across both visual and auditory stimuli and can easily integrate these dimensions (de Hevia et al., 2014b)

Another study involving 7-month-old infants revealed a preference for left-to-right sequences of increasing dots over right-to-left sequences. This preference was observed regardless of the infants' habituation conditions—whether they were habituated to increasing sequences from right-to-left or decreasing sequences from left-to-right (de Hevia et al., 2014a) (Fig. 9).

These studies support the notion that infants, even in their earliest months, exhibit a spatial mapping of numerosity that follows a left-to-right configuration. However, evidence suggesting that this spatial mapping specifically links smaller numbers to the left side and larger numbers to the right side only emerges a year later.

Indeed, the first compelling evidence of a SNARC-like effect in preverbal infants comes from research conducted by Bulf and colleagues using the Posner Paradigm. They discovered that 8-month-old infants looked more quickly toward a target on the left when it came after a small numerosity and toward a target on the right when it came after a large numerosity (Bulf et al., 2015) (Fig.10).

More recently, de Hevia et al. (2017) also provided convincing evidence of the mental number line in newborns. In their study, neonates aged 0 to 3 days were habituated to an image of a long line with many sounds or a short line with few sounds. Following this habituation, the infants were shown two lines on either side: shorter lines associated with few sounds or longer lines associated with many sounds. The newborns looked longer at the left side if the line and sound were shorter and fewer than during habituation, and at the right side if they were longer and more numerous.

In a follow-up experiment, researchers tested infants using auditory stimuli with sounds varying in duration rather than quantity. The infants did not show differential looking behavior toward either side. Similar results occurred when visual stimuli were used alone without auditory cues.

In subsequent experiments, the researchers reintroduced numerical elements into the auditory stimuli, ensuring that the number of sounds varied while the duration remained constant. Under these conditions, the SNARC effect reemerged, indicating that continuous cues alone were insufficient to elicit the effect. This finding underscores the importance of numerical stimulation for the manifestation of the SNARC effect in newborns.

Similar results have been confirmed by research conducted by Di Giorgio and colleagues in 2019. They tested twenty-four newborns at approximately 51 hours of age using a computer-based task. Infants were habituated to a specific number by showing them images with 12 dots on both the right and left sides. Subsequently, they were presented with images featuring either a smaller set (4 dots) or a larger set (36 dots).

After habituation, infants looked longer to the left for 4 dots and to the right for 36 dots, demonstrating a left-to-right oriented spatial numerical association. In two subsequent experiments, Di Giorgio and colleagues habituated one group of infants to the numerosity 4 and another group to the numerosity 36. When presented with two images depicting 12 dots on the left and right, infants habituated to 4 looked longer at the image on the right, while those habituated to 36 looked longer at the image on the left. These two experiments demonstrate the relativity of the newborns' MNL, which is a fundamental feature of the adult MNL. Specifically, the numerosity 12 was associated with the left if the habituation number was larger (36) and with the right if it was smaller (4). This suggests that the number-space association in newborns is based not on the absolute value of the numbers, but on their relative contextual range. Furthermore, the stimuli were controlled for perimeter and area, ensuring that infants did not rely on these features. This supports the idea that newborns spontaneously use abstract numerical cues just a few days after birth, in line with earlier findings by Izard and colleagues in 2009 (Izard et al., 2009).

These studies suggest that the mental number line may not be solely a product of cultural factors but could instead have biological underpinnings related to pre-linguistic processes (Adachi, 2014; Bulf et al., 2015; De Hevia et al., 2014; Drucker & Brannon, 2014; Lourenco & Longo, 2010; Rugani et al., 2014; Rugani et al., 2015b, 2016; Vallortigara, 2018). While its orientation might be influenced by cultural factors, the fundamental roots of the mental number line appear to be biological.

To further investigate the influence of cultural and biological factors in the development of the MNL, Eccher and colleagues (Eccher et al., 2023) recently conducted experiments involving three distinct cohorts representing varying cultural backgrounds and age groups: Italian adults, Italian preschoolers, and Himba adults. Evidence of a SNARC effect was exclusively observed among Italian adults when instructed to explicitly sequence numerosities. However, when tested during a implicit numerosity comparison task, a left-to-right bias was identified also in preschoolers and the Himba population (Eccher et al., 2023).

The authors proposed then a new hypothesis: the SNARC effect might not represent a singular phenomenon but rather could be divisible into two distinct components. The first component is universal and innate, marked by a left-to-right bias, which emerges in implicit tasks devoid of explicit numerical judgments and does not necessitate cultural inputs. The second component is delineated as acquired and culturally dependent, emerging prominently during explicit tasks within literate populations.

They proposed the coexistence of these two components of the SNARC effect, which was also evidenced by their findings: employing implicit measures to explore automatic behaviors, they unveiled a left-to-right SNARC effect among both preschoolers, adult members of the Himba community, and Italian adults. Nonetheless, the SNARC effect emerged solely among literate adults when explicit instructions were provided for numerical mapping. This accentuates the importance of task guidelines in delineating the distinct components of the SNARC phenomenon (Eccher et al., 2023).

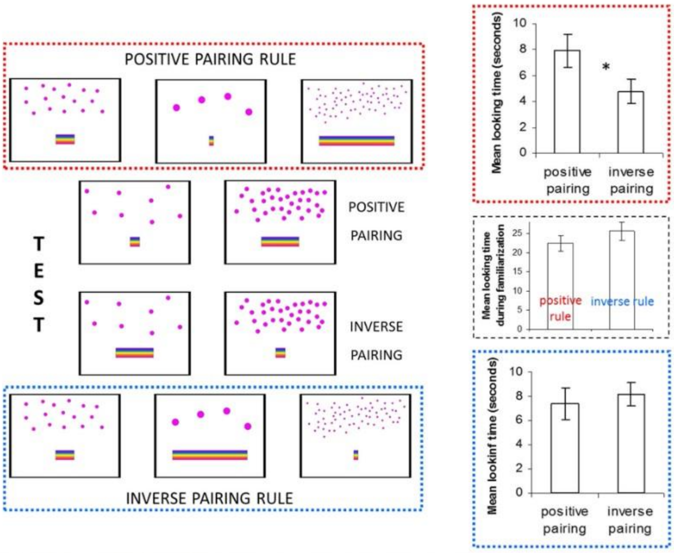


*__Figure 8.__ Task protocol and behavioural performance by de Hevia & Spelke, 2010. Eight-month-old infants were introduced to either a positive (red) or inverse (blue) relationship between number and line length. During testing, infants exposed to the positive rule looked longer at new examples that followed this rule, while those exposed to the inverse rule showed no preference in looking time. Image from Rugani & de Hevia, 2017*

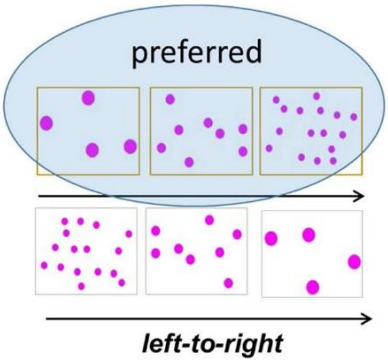


*__Figure 9__. Paradigm used in de Hevia et al.,2014a. Seven-month-old infants, when exposed to numerical sequences that either ascend or descend from left to right, demonstrate the capacity to recognize the order, displaying a preference for sequences that increase in numerosity from left to right. Image from Rugani & de Hevia, 2017*

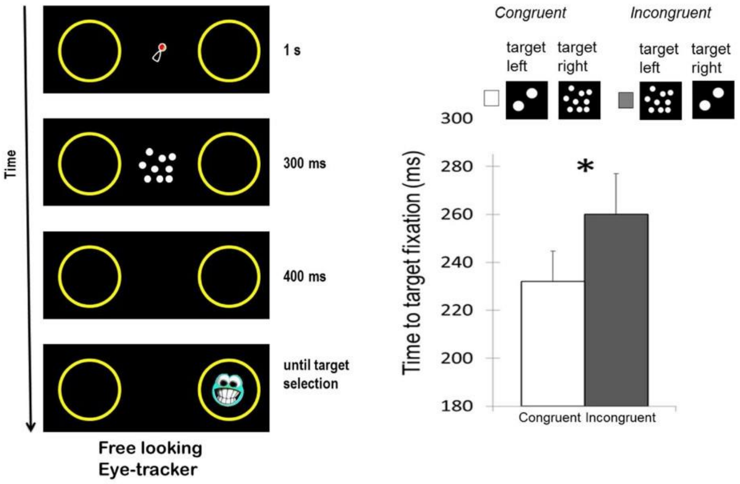


***Figure 10.*** *Posner-like Paradigm used by Bulf et al., 2015. (Left Panel) Example of a congruent trial in which a large central numerosity is succeeded by a target appearing on the right side of the screen. Infants tend to respond more rapidly to these congruent trials (where a small numerosity is followed by a left target and a large number by a right target) in comparison to incongruent trials, where the target and numerosity placement are mismatched (a small number followed by a right target and a large number followed by a left target). Image from Rugani & de Hevia, 2017*

## *Number-Space Association in non-human animals*

In recent years, several studies have been conducted to investigate whether the mental number line is also present in non-human animals. A pioneering study on this topic was conducted by Rugani, Regolin, and Vallortigara in 2007 using five-day-old chicks. Initially, the chicks were trained to peck at either the third, fourth, or sixth hole in a row of 10 holes arranged on a panel. All groups of chicks successfully completed the task, revealing their capability to understand ordinal information (Rugani et al., 2007). Subsequently, another group was trained to peck the fourth dot in a series of 10 identical and equidistant dots arranged vertically. Following this, the row of dots was rotated by 90 degrees, becoming horizontal, and the chicks were asked to peck the fourth dot. The chicks significantly preferred to peck the fourth dot from the left rather than the right (Rugani et al., 2007), indicating a tendency to count from left to right. However, the same result was not confirmed when the birds could rely only on numerical cues, suggesting that they show a left-to-right bias only when numerical and spatial information are linked (Rugani et al., 2011).

The same study, with some adjustment, was successfully replicated on adult Clark’s nutcrackers (Rugani et al., 2010) and rhesus macaques (Drucker & Brannon, 2014). The results obtained from studies on birds can be interpreted as a reminiscent of the human mental number line, but alternative explanations have also been proposed. For instance, some authors (Rugani & de Hevia, 2017) have hypothesized that the bias towards the left space may be partly attributed to the phenomenon known as 'pseudoneglect,' found in humans (Albert, 1973), in birds (Regolin, 2006) and amphibians (pseudoneglect-like phenomenon, Vallortigara et al., 1998).

This phenomenon appears to exhibit a greater allocation of attention in the left hemispace (Diekamp et al., 2005) and a tendency to overestimate the left side of space in specific contexts (Regolin, 2006). Moreover, in chicks spatial cues are processed in the right hemisphere of the forebrain, which enhances the preference for allocating attention to the left hemifield (Tommasi et al., 2003; Tommasi & Vallortigara, 2001; Rashid & Andrew, 1989; Regolin et al., 2005).

However, the phenomenon of pseudoneglect and the asymmetry in the representation of spatial information have not yet been described in other species that exhibit a preference for 'counting' from left to right, and therefore do not currently provide an explanation for the presence of this bias in non-human animals. Moreover, in the study by Drucker and Brannon, rhesus macaques showed a left-to-right bias while performing the same task as Rugani and colleagues (2007) with chicks in a computerized version, even when the target was located in the right hemifield (Drucker & Brannon, 2014).

These studies demonstrate that in tasks requiring the identification of a target based on its ordinal position, some species exhibit a tendency to associate numbers with spatial locations. However, they fail to show a specific association of larger numbers with the right side of space and smaller numbers with the left, as observed in the human mental number line. Additionally, these studies do not reveal another crucial aspect of number-space association: numerical relativity concerning the contextual range. For example, the number 5 is considered small within the interval between 5 and 10, but large within the interval between 0 and 5.

The first convincing demostration about a bidirectional mental number line and the relativity of the numbers in a spatial configuration come from a study carried out some years later by Rugani and collegues (Rugani et al., 2015b).

In this investigation, three-day-old chicks were trained to navigate around a panel featuring five dots (the target) to obtain a food reward. After familiarization with the target number, two panels with numerosities either greater or smaller than the target were presented, positioned to the left and right of the chicks' starting point. The chicks exhibited a tendency to approach the left panel when the numerosity was smaller than the target (e.g., 2 dots) and to approach the right panel when the numerosity was larger (e.g., 8 dots), reflecting the left-to-right orientation of the mental number line. Furthermore, when other chicks were trained with panels displaying 20 dots, they chose the left panel when 8 dots were shown and the right panel when 32 dots were shown, demonstrating the relative nature of the mental number line (in the first test, chicks perceived the numerosity 8 as large, while in the second test, they considered it small; Rugani et al., 2015b).

Subsequently, Beran and collegues (2019) attempted to replicate this experimental design with 5 rhesus macaques and 14 capuchin monkeys using a computerized task. However, their findings did not corroborate those of the previous study (Beran et al., 2019). The subjects were instructed to use a joystick to complete various game-like tasks. In one task, the monkeys were familiarized with a screen displaying five dots (the target) and were required to choose between a greater (8 dots) or lesser (2 dots) set of dots positioned to their right and left. The study found no clear evidence of a spatial bias; only a limited SNARC effect was observed in 12 out of 19 subjects when selecting between two distinct arrays of dots positioned to their right or left, suggesting a spatial representation of quantities in these species without a consistent orientation. In this study, however, responses during the test trials were consistently rewarded with a single pellet, whereas in the study by Rugani and colleagues (Rugani et al., 2015b), the chicks received no rewards during the test trials. These differences in outcomes may be also attributed to the variations in experimental manipulations.

Recently, Rugani and collegues (2024) provided new and convincing evidence for a spatially oriented mental magnitude line in rhesus macaques. In their study, monkeys were tasked with remembering the location of a target within arrays of dots, which varied in size from 2 to 10 dots. The results revealed that the monkeys exhibited enhanced accuracy in recalling left-side targets with 2-dot arrays and right-side targets with 6- or 10-dot arrays. Furthermore, when the researchers swapped out the largest dot array (10-dot array) for a long bar —aiming to assess whether the monkeys could generalize their learned rule from discrete dot arrays to a continuously extended stimulus—performance on right-side targets improved. This last result highlighted the fact that in macaques, spatial mapping is widespread across all magnitudes: the monkeys demonstrated spatial biases when presented with both clusters of dots and continuous lines (Rugani et al., 2024).

Other investigations into non-human animals have also been performed. Among apes, gorillas and orangutants were tested in a quantity discrimination task on a touch screen computer showing evidence for a SNARC effect in these species, despite individual variations in the direction of the phenomenon (Gazes et al., 2017). Chimpanzees were trained by Adachi in 2014 to touch arabic numbers, from 1 to 9, in ascending order on a touch screen. Then, in the testing phase, subjects had to touch the number 1 or 9 that were shown to the left or the right on the screen. The results showed that chimps were faster to touch the number “1” when it was positioned at the left and the “9” when it was positioned on the right (Adachi, 2014). While this study is intriguing, it does not necessarily indicate that chimpanzees were processing numerical magnitudes. Instead, their behavior may reflect an alternative cognitive process, such as sequential reasoning, which involves understanding ordered sequences rather than numerical values.

Then, the authors Johnson-Ulrich and Vonk tested American black bears (*Ursus americanus*), Western lowland gorillas (*Gorilla gorilla gorilla*), and humans in a quantity discrimination task where subjects had to choose the larger or the smaller array of dots presented at left and at right of a computer monitor. They found some evidence of SNARC-like effects in all species, even though the orientation of performance was inconsistent among individuals and response times also differed across conditions and subjects (Johnson-Ulrich & Vonk, 2018).

Lastly, evidence of a SNARC effect recently emerged also in arthropods: Giurfa and collegues trained honeybees to associate a training stimulus (white square with dots) placed in the middle of a wall at the end of a wooden box with a sucrose reward. Then, they faced the honeybees with 2 stimuli depicting the same number of dots and they found out that bees choose significantly more at left when the test stimuli detected less dots than the training stimulus and at right when the test stimuli was larger than the training one (Giurfa et al., 2022).

Overall, these studies indicate a possible evolutionary basis for SNARC-like effects, suggesting that such spatial-numerical associations extend across different species. However, the exact direction of this effect and the mechanism behind it remains a topic of ongoing debate.

## *Mechanisms underlying the association between numbers and space*

Regarding the neurobiological mechanisms underlying the associations between numbers and space, clear explanations have yet to emerge. While some evidence has been found in our species, it only provides partial insights into the behavioral number-space association.
One of the earliest hypotheses regarding the link between space and numbers was proposed by Walsh in 2003 with "A Theory Of Magnitude (ATOM)". This theory posits that space, numbers, and even time are components of a general magnitude system, which is neuroanatomically anchored in the neurons of the inferior parietal cortex (Walsh, 2003). Indeed, this brain region has been shown to become active during spatial, numerical, and temporal processing (Cochon et al., 1999; Dehaene et al., 1999 ; Harvey et al., 2015; Rao et al., 2001; Simon et al., 2002), suggesting a shared neural substrate for these dimensions.
Behavioral studies have indeed demonstrated that humans not only associate numerical quantities with spatial dimensions but also link temporal magnitudes—such as music tempo (Prpic et al., 2013), pitch level (Pitteri et al., 2017 ; Rusconi et al., 2006), and temporal lenght (Bonato et al., 2012 ; Vallesi et al., 2008)—with space. Evolutionarily, this association may be explained by the co-variation of numerical and continuous cues in nature, suggesting that a unified system for representing both quantities would be advantageous for the brain (Rugani & de Hevia, 2017).
Stronger evidence for the neurobiological basis of the association between space and numbers comes from Harvey et al. (2013), who identified neural populations in the human parietal cortex specifically responsive to small numerosities (less than 7). The activity in the area is organized topographically, with favored numerosities increasing from medial to lateral regions of the parietal cortex (Harvey et al., 2013). However, this topographic map has been observed consistently only in the right hemisphere and has not been identified in other species, offering limited insights into the behavioral number-space association (Harvey et al., 2013; Fig. 11). In contrast, while neurons tuned to numerical values have been found in the intra-parietal cortices of macaques (Nieder & Miller, 2004), and some are responsive to both number and length (Tudusciuc & Nieder, 2007), evidence for a similar topographic map in non-human primates remains lacking.

Another potential explanation for the phenomenon comes from the observation of overlapping neural activity in the lateral and ventral intraparietal areas of the brain, which are involved in the processing of both numerical and spatial information in primates. This suggests that spatial and numerical cognition may share joint neural pathways (Drucker & Brannon, 2015; Hubbard et al., 2005). However, while this finding highlights the correlation between continuous variables like space and numerical variables, it does not address the specific left-to-right orientation of the mental number line (Vallortigara, 2018).

The hemispheric lateralization of the brain, a shared and ancestral trait among vertebrates, has also been posited as a primary factor for the association between numbers and space and its orientation (de Hevia et al., 2012; 2014b; Rugani et al., 2015a). Indeed, the right hemisphere plays a dominant role in tasks involving visuospatial and numerical processing in some species (Emerson & Cantlon, 2015; Hyde et al., 2010; Rugani et al., 2015a; Rugani et al., 2016), potentially leading to a preference for the left hemifield and thus contributing to the phenomenon of counting from left to right.

Expanding on the idea of hemispheric lateralization, Giorgio Vallortigara recently proposed the "motivational hypothesis" as a potential explanation for the orientation of the SNARC effect (Vallortigara, 2018). This hypothesis is based on the so-called "valence hypothesis" (Davidson, 2004), which posits that the two hemispheres are specialized in processes with different "valence". Specifically, the anterior regions of the left hemisphere are specialized in approach processes, and thus are linked with positive emotions, while those of the right hemisphere are specialized in withdrawal processes and consequently associated with negative emotions. Evidence of this hemispheric specialization has been found in both humans and non-human animals, such as dogs, who wag their tails more to the right for positive stimuli and to the left for negative ones (Quaranta et al., 2007).

Applying this hypothesis to the domain of number-space association, Vallortigara suggests that for an appetitive stimulus, such as a preferred food, larger quantities can be considered as inherently better than smaller ones. Consequently, a shift from a smaller to a larger number of appetitive stimuli would predominantly activate the left hemisphere, thereby enhancing activation of the contralateral hemispace, the right. Conversely, a shift from a larger to a smaller number of appetitive stimuli would lead to predominant activation of the right hemisphere and its contralateral hemispace, which is the left (Vallortigara, 2018) (Fig.12). Although this hypothesis has the advantage of being empirically testable, it has not yet been demonstrated to date.

Lastly, Felisatti and collegues (2020) proposed the brain's asymmetric frequency tuning (BAFT) hypothesis based on the brain hemispheric specialitation in visual feature processing (Felisatti et al., 2020a,b). The BAFT is based on the evidence that the right hemisphere (left visual field) is specialized in extracting low raw spatial frequencies (SF) content, while the left hemisphere (right visual field) in high raw SF (Christman, 1989; Fink et al., 1996; Peyrin et al., 2003; Piazza & Silver, 2017). Since small arrays of objects/dots are represented by lower spatial frequencies ranges than larger arrays, the right hemisphere would be activated for smaller quantities, leading to a behavioral response on the left. Conversely, larger arrays, which are associated with higher spatial frequencies, would activate the left hemisphere, resulting in a right-side response for larger quantities. This differential activation pattern may explain both the directionality and the relative nature of the SNARC effect. Indeed, the authors propose that the ability to discriminate between low and high spatial frequencies (SFs) depends on the SF range present in a given image (Flevaris et al., 2011; Piazza & Silver, 2017). This is analogous to how number-space associations are influenced by the numerical range in which a given number falls (Dehaene et al., 1993). However, this hypothesis is not supported by the evidence showing that the right/left bias occurs only when, after habituation to a numerical stimulus, there is a shift to a different numerical quantity (either greater or smaller than the target stimulus). No bias is observed when there is no shift, and the numerical quantity remains the same (Rugani et al., 2020). These findings suggest that it is not spatial frequency per se that drives the lateralization bias, but rather the change in spatial frequency, as predicted by the motivational hypothesis (Vallortigara, 2018)

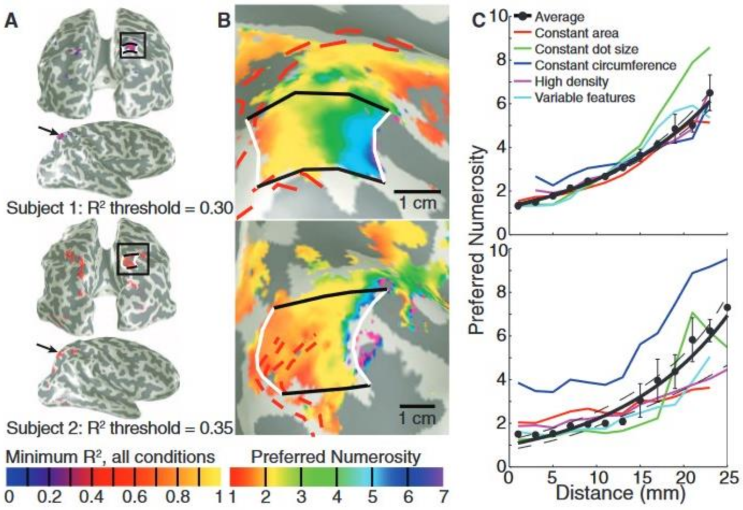


*__Figure 11.__ Topographic mapping of numerosity in the right parietal cortex of human brain (A) reveals a region with neurons tuned to quantity across different stimuli (B). Preferences for numerosity increase progressively from the medial to lateral sides of this area (C). Description and image from Harvey et al., 2013*

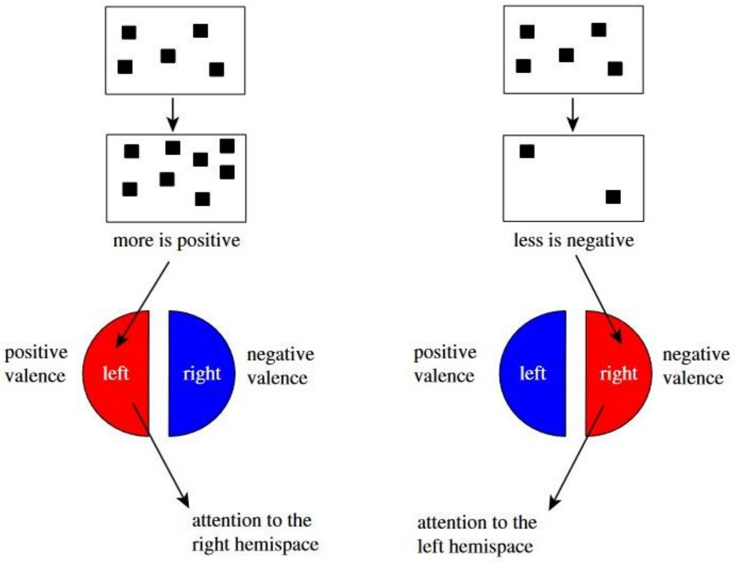


*__Figure 12.__ Schematic representation of the motivational hypothesis proposed by Giorgio Vallortigara (Vallortigara, 2018). Valence theories suggest that the two brain hemispheres contribute differently to processing emotions, with the left hemisphere associated with positive valence (approach) and the right with negative valence (avoidance). If increasing numerosity activates the left hemisphere and decreasing numerosity activates the right, attention would naturally shift to the opposite side of space. This could explain why smaller magnitudes are often linked to choices on the left side and larger magnitudes to the right.*

## *Macaques as a model to study Number-Space Association*

Macaques represent a good model for investigating the phenomenon of number-space association due to their due to their human-like social, cognitive, emotional, physiological, and genetic traits (Phillips et al., 2014; Rosati et al., 2016; Sallet, 2022; Wang et al., 2020; Xue et al., 2016).

Additionally, several behavioral studies provided evidence of numerical competence in macaques, demonstrating their ability to flexibly distinguish between different numerosities, suggesting a numerical representation based on absolute values (Cantlon & Brannon, 2006; Merten & Nieder, 2009; Nieder, 2012). Macaque's performance also reveals patterns similar to those found in human numerical estimation, such as the distance effect and the size effect (Beran, 2008; Brannon & Terrace, 2002; Cantlon & Brannon, 2006), indicating the presence of a shared common system for estimating quantities.

Neurobiological research has also identified number neurons in macaques, specifically in the frontal, parietal, and temporal associative cortices, which selectively respond to preferred numerosities (Nieder, 2016; Nieder et al., 2002; Nieder & Miller, 2003; Okuyama et al., 2015; Viswanathan & Nieder, 2015). Moreover, similar to humans, there is neural overlap between numerical and spatial processing in the intraparietal sulcus (IPS) of monkeys (Hubbard et al., 2005), suggesting that there may be a connection between the two types of information.

However, there remains some contention regarding the orientation of number-space mapping in these species, with certain studies reporting a left-to-right bias (Drucker & Brannon, 2014; Rugani et al., 2024), while others have found limited evidence supporting it (Beran et al., 2019).

## *Aim of this thesis*

Although some hypotheses have been suggested regarding the origin and the mechanism underlying the Number-Space Association (NSA), several questions remain unresolved. This thesis aims to offer evidence on the origin and the underlying mechanisms of the SNARC effect in macaques.

Specifically, the Experiment I and II are aimed to verify the occurrence of a left-to-right MNL in macaques. Since numerical skills in non-human animals have evolved within ecological contexts, we used a spontaneous food-related task to ensure ecological validity and investigate automatic behaviors. This approach aligns with the hypothesis proposed by Eccher and colleagues, which suggests that the innate component of the SNARC effect emerges in implicit tasks devoid of explicit numerical judgments (Eccher et al., 2023).

In line with this, investigations conducted on capuchin monkeys demonstrated higher performance in quantity discrimination tasks involving food compared to token-based tasks, likely due to the heightened relevance of food stimuli over tokens (Addessi et al., 2007).

Subsequently, we investigated the mechanisms underlying the number-space association in macaques. We tested (Experiment III, IV and V) the motivational hypothesis proposed by Vallortigara (2018), which is advantageous due to its ease of empirical verification. Specifically, we aimed to assess whether an aversive stimulus could modify the Mental Number Line in macaques. To this end, we opted for a spontaneous task involving both negative and neutral stimuli, using food-based and computerized versions of a similar sets of stimuli.

# Chapter III. *Studies*

## Study 1. Investigating the SNARC effect in *Macaca mulatta* and *Macaca fascicularis*

### Abstract

According to the mental number line (MNL), humans associate numbers into the space in a left-to-right spatial configuration, with smaller quantities represented preferentially in the left-sided space and vice versa. The orientation of the MNL in our species can also vary and be affected by cultural habits, such as the direction of reading and writing. However, a growing number of studies on pre-verbal infants and non-human animals posit that the orientation of MNL can also have biological bases. To deepen such biological predisposition, here we investigated the occurrence of a number-space mapping in a non-human primate species. We involved 17 macaques from two different species (7 *Macaca mulatta* and 10 *Macaca fascicularis*) in two different behavioral experiments by using a food-related testing procedure. In Experiment I, the subjects were administered with six possible pair-wise combinations (1x1, 2x2, 4x4, 8x8, 16x16, 24x24) of the same quantity of pieces of food presented in the left and right space in front of the monkey. Results showed that monkeys reported a higher percentage of left choices for smaller quantities and a higher percentage of right choices for larger quantities. In Experiment II, macaques were familiarized with the pair-wise combination 4x4 ("Habituation small") or 16x16 ("Habituation large") and then tested with a novel pair trial (respectively 16x16 for "Habituation small" and 4x4 for "Habituation large"). Monkeys showed a higher percentage of left choices in the novel trial when it was preceded by the "habituation large" phase. In contrast, individuals showed a higher percentage of right choices in the novel trial preceded by the "habituation small" phase. These findings seem to confirm the biological and evolutionary roots of MNL, probably representing a universal cognitive strategy in the animal kingdom.

### Introduction

To this day, the debate over whether the orientation of the mental number line is driven by cultural factors, biological factors, or both remains controversial. Some studies suggest that the direction of the MNL develops in response to educational and cultural influences (Patro et al., 2016; Shaki et al., 2009). In contrast, other research, particularly involving preverbal infants and non-human animals, indicates that the MNL might be a universal cognitive strategy rooted in prelinguistic factors (Adachi, 2014; Bulf et al., 2015; De Hevia et al., 2014; Drucker & Brannon, 2014; Eccher et al., 2023; Lourenco & Longo, 2010; Rugani et al., 2014; Rugani et al., 2016; Vallortigara, 2018).

In macaques, although there is evidence of a certain mapping of numbers in the space (Beran et al., 2019; Drucker & Brannon, 2014; Rugani et al., 2024), the existence of an oriented left-to-right mental number line remains unclear. Drucker and Brannon (2014) observed that macaques, after being trained to select the fourth dot from the bottom in a vertical array of five elements, subsequently chose the fourth from the left when the same array was presented horizontally. This behavior suggests a left-to-right spatial mapping of numerical information. However, it remains unclear whether the monkeys were specifically selecting the fourth from the left or the second from the right, raising the possibility of a right-to-left bias instead of a left-to-right mapping. A left-to-right orientation in macaques was recently reported by Rugani and colleagues (2024) using a different experimental setup. In their study, two macaques were tasked with remembering the position of a target within an array that varied in numerosity (ranging from 2 to 10 dots). As the number of dots increased, the monkeys' performance shifted, with greater accuracy initially on the left side, then improving on the right. However, this bias was not consistent across all conditions (2, 4, 6, and 10 dots), and the study was conducted with a limited range of numerosity and only on two subjects, highlighting the need for a larger sample. Moreover, Beran and colleagues (2020) replicated Rugani et al.'s (Rugani et al., 2015b) two-choice discrimination paradigm in a computerized version. In their study, the monkeys were habituated to a central target (array of dots) displayed on a touchscreen before being tested with either a smaller or larger numerosity presented to the left and right of the monitor. In contrast to Rugani et al.'s findings with chicks, which demonstrated a consistent tendency for the animals to select the set on the left when it was smaller and on the right when it was larger than the target, the monkeys did not exhibit a similar selection pattern. However, a spatial bias was observed in 12 out of 19 subjects when they were asked to choose between two distinct arrays of dots positioned to their right or left. Notably, some monkeys performed better when the smaller set was on the left, while others showed improved performance when it was on the right, suggesting that the direction of the bias was not clearly defined. Given the varying results observed in previous studies and the necessity to further investigate the presence of this phenomenon in macaques, we designed and implemented two food-related behavioral tasks aimed at exploring their spatial mapping of numerical information.

In the first experiment, we explored spontaneous number-space associations by presenting macaques with equal quantities on the left and right, ranging from 1 to 24, without introducing a reference number or prior habituation. This design aimed to assess the monkeys' inherent predispositions to associate numerical values with spatial positions, investigating whether a natural tendency exists to map smaller or larger numbers to the left or right hemispace, respectively.

Conversely, the second experiment employed a structured habituation-test paradigm involving only two specific numerosities. During the habituation phase, macaques were repeatedly exposed to a reference numerosity, establishing a baseline for numerical magnitude. In the subsequent test phase, they were presented with numerosities either greater or smaller than this reference, allowing us to examine their relative numerical spatial biases. This paradigm was first successfully employed by Rugani and colleagues (Rugani et al., 2015b) with chicks, and later replicated in bees (Giurfa et al., 2022) and human newborns (Di Giorgio et al., 2019), yielding results that suggest a left-to-right spatial mapping of numerical information in these species. Beran and colleagues (Beran et al., 2020) also adapted this paradigm in a computerized version for macaques, but did not observe the same pattern. One possible explanation for the differing outcomes could be the variations in experimental manipulations: for instance, test trials were reinforced in Beran et al.'s study (Beran et al., 2020), whereas they were conducted in extinction in Rugani et al.'s study (Rugani et al., 2015b).

In our study (Experiment II), we sought to replicate this approach with macaques, introducing some modifications. First of all, given that numerical abilities likely evolved in natural contexts, we aimed to create a more ecologically valid setup by using food as a stimulus. This approach was intended to increase the monkeys' motivation and engagement with the task, reducing potential distractions and improving task performance. Presenting real food as a stimulus, in contrast to other representations, may enhance attentional focus and is consistent with findings that non-human primates may have a more salient representation of numerosity when it involves food, compared to other types of stimuli (Addessi, 2014 ; Beran et al., 2005). In contrast to prior studies (Di Giorgio et al., 2019; Giurfa et al., 2022; Rugani et al., 2015b), which employed a centrally presented target, our study permitted the monkeys to make lateral choices during the habituation trials. This methodological modification was pivotal in enabling a comparative analysis of responses across both habituation and test trials. By adopting this approach, we aimed to investigate how the monkeys' choices shifted from the final phase of habituation to the novel phase, when they were presented with a different numerosity.

The two paradigms employed in Experiments I and II were designed to provide a comprehensive assessment of the mental number line in macaques across different cognitive contexts: one focusing on numerical perception within a range, without habituation to a specific target, and the other on relative numerical comparison, anchored by a reference point. We hypothesize that in both conditions, macaques will exhibit a consistent pattern of associating smaller numbers with the left side and larger numbers with the right side, supporting the existence of a spatial-numerical association akin to the SNARC effect observed in humans.

## *Experiment I.*

### Subjects

In the current experiment, we tested 17 macaques, 7 rhesus macaques (*Macaca mulatta*) and 10 crab-eating macaques (*Macaca fascicularis*), housed at the Institut des Sciences Cognitives Marc Jeannerod (Lyon, France). The age of the monkeys ranged from 3 to 14 years (mean age = 5.38; sd = 3.32) (see Table 1). The subjects were housed in colony cages with one or more partners. During the test they were isolated in a testing cages (220 cm x 70 cm X 115 cm) and they were free to move without any hindrance. Fresh fruit, pellet and nuts were provided daily. Water was available ad libitum.

All housing and procedures used in this research complied with the current guidelines in the matter of care and use of laboratory animals (European Community Council Directive No. 86–609), and were authorized by our local ethics board (03.10.18) and the French Ministry of Research (10.10.18; authorization no. 15091_2018071014483295_v2).

| Subject | Species | Sex | Age (years) | Task |
|---|---|---|---|---|
| **ZP22 - Miaou** | *Macaca mulatta* | M | 5 | Experiment I |
| **ZP21 - Machupichu** | *Macaca mulatta* | M | 5 | Experiment I |
| **ZP15 - Magnesium** | *Macaca mulatta* | M | 5 | Experiment I |
| **ZP16 - Mendiant** | *Macaca mulatta* | M | 5 | Experiment I |
| **ZP14 - Maboul** | *Macaca mulatta* | M | 5 | Experiment I |
| **Kori** | *Macaca fascicularis* | M | 5 | Experiment I and II |
| **Oscar** | *Macaca fascicularis* | M | 6 | Experiment I and II |
| **Ciro** | *Macaca fascicularis* | M | 3 | Experiment I and II |
| **Genny** | *Macaca fascicularis* | M | 3 | Experiment I and II |
| **Platon** | *Macaca fascicularis* | M | 4 | Experiment I and II |
| **Diogéne** | *Macaca fascicularis* | M | 4 | Experiment I and II |
| **Scooby** | *Macaca mulatta* | M | 14 | Experiment I and II |
| **Samurai** | *Macaca mulatta* | M | 13 | Experiment I and II |
| **Yogi** | *Macaca fascicularis* | M | 3 | Experiment I and II |
| **Edward** | *Macaca fascicularis* | M | 3 | Experiment I and II |
| **Morty** | *Macaca fascicularis* | M | 3 | Experiment I and II |
| **Rick** | *Macaca fascicularis* | M | 3 | Experiment I and II |

***Table 1 .*** *Table of information (Names, Species, Sex, Age) of experimental subjects in Experiment I and/or II.*

### Apparatus

The apparatus consisted of a testing cage with a frontal PVC plexiglass panel (122 cm x 46 cm x 3 cm). The PVC panel had two holes at the bottom, one on the left and one on the right, each positioned 10.5 cm from the border (Figure 13.a). A shelf (25 cm x 83 cm x 1 cm) was attached to the cage to allow the experimenter to place two food containers in front of the monkey. The food containers comprised two test tube holders (8 cm x 12.5 cm x 2 cm), positioned on the left and right parts of a tray (a wooden support measuring 12 cm x 38 cm x 2 cm), equidistant from the board (Figure 13.b). Each test tube holder contained 24 holes (diameter 1.8 cm), spaced 2 mm apart. A metal stick was attached to the shelf to designate the point from which the monkeys could reach the food. This spatial index was used by the experimenter to place the tray within the monkey's "reachable space."

(a) (b)

***Figure 13**. **(a)** Apparatus: Testing cage with a PVC plexiglass. PVC had two holes at the bottom through which the monkey could reach food. **(b)** the food boards.*

### Experimental Procedure

The experimental procedure was adapted from Rugani and collegues (2015), with several modifications. We designed an equal comparison quantity task where numerical targets were represented by pieces of food instead of dots on a panel, ensuring the stimuli were ecologically relevant to the monkeys. We chose to exclude a training phase with a reference number in order to observe the subjects' spontaneous behavioral responses to varying quantities within a numerical range.

Each monkey was pseudorandomly presented with six possible pair-wise combinations of the same quantity of food pieces in the left and right food boards: 1 x 1, 2 x. 2, 4 x 4, 8 x 8, 16 x 16 and 24 x 24. The food pieces were raisins, placed by the experimenter inside the holes of both tube racks (Figure 14.a).

For each trial, the experimenter arranged the food containers with her back turned to the monkey, preventing the subject from viewing the contents before the trial began. The complete experimental procedure is illustrated step-by-step in Figure 14.b.

If the monkey did not direct its gaze towards both food containers within ten seconds, the trial was terminated, and the tube racks were removed. Each subject underwent an experimental session consisting of thirty trials, with each of the six food pairwise combinations presented five times in a pseudo-random order.

As soon as the subject chose, reached, and grasped one raisin from one of the two (left or right) food containers, the experimenter removed the containers. Limiting the reward to only one piece of food per trial was intended to prevent satiation and ensure that the subject maintained sufficient motivation to continue and complete the experiment. If the monkey did not take any food within ten seconds, the experimenter removed the tube racks.

The procedure was repeated for 30 subsequent successful trials, defined as trials where the monkey looked at both food containers and reached for a piece of food from one of them. The subjects did not undergo any training sessions but only a familiarization phase to become comfortable with the experimental apparatus. Each subject was tested individually and isolated from the group during the experiment.

All experimental sessions have been video recorded through one camera (Digital Videocamera Panasonic HC V-180 Full-HD optical zoom 90×), allowing offline data elaboration.

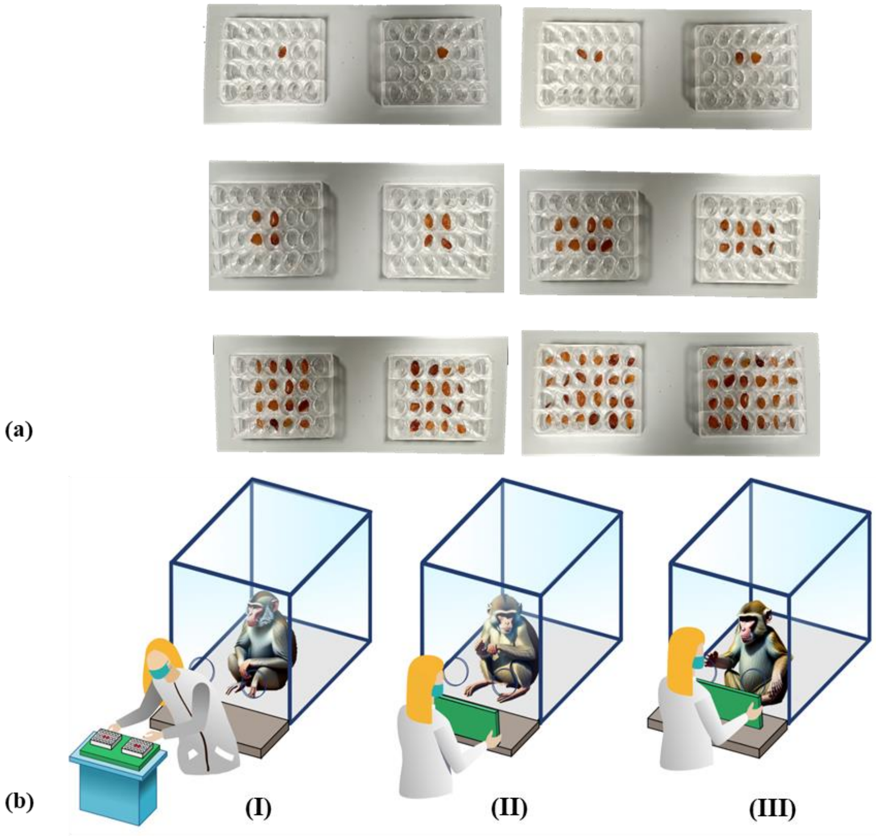


*Figure 14.* ***(a)*** *The six possible pair-wise combinations of the same quantity of pieces of food placed in the left and in the right food container.* ***(b)*** *Illustration of the experimental procedure: (I) Experimenter prepares food containers with back turned to monkey; (II) Experimenter shows tray out of reach, waiting for monkey to look; (III) Experimenter brings containers within reach, monkey chooses left/right and takes one raisin.*

## Data Elaboration

For each animal, the experimental session was coded offline using Avidemux 2.7.8 Release by the experimenter. A second researcher coded a random 20% of videos to assess reliability. The reliability scores between coders were very high (all $\kappa > 0.8$). For each experimental trial, the following variables were coded: the number of stimuli presented to the monkey (1, 2, 4, 8, 16 or 24) the choice made by the subject (right or left food container) and the hand used to grasp the food. Trials in which the monkey did not look at both food containers (left and right) were classified as “inaccurate” and were excluded from the analysis. This exclusion was essential to prevent potential biases in the results, as it was uncertain whether the monkeys had adequately attended to and assessed both food quantities in these trials. One subject was excluded from the analysis due to its biased choice (right choices) throughout the entire task.

The six possible pair-wise combinations of the same quantity of pieces of food (1 x 1, 2 x 2, 4 x 4, 8 x 8, 16 x 16, 24 x 24) were classified as follows: 1 x 1, 2 x 2, 4 x 4, and 8 x 8 were considered "small quantities," whereas 16 x 16 and 24 x 24 were defined as "large quantities."

This classification is based on cognitive principles suggesting that number perception is relative to the numerical range in which quantities are presented. This relativity is a well-established feature of the Mental Number Line (MNL) in both humans (Dehaene, 2011; Di Giorgio et al., 2019) and non-human animals (Rugani et al., 2015b). For example, Rugani et al. (2015) found that chicks trained with the number 20 chose 8 to the left, while those trained with 5 chose 8 to the right, illustrating how numerical perception shifts based on context.

Given the broader range of numbers presented in our experiment (up to 24), we classified numbers up to 8 as small and 16 and 24 as large. This classification aligns with Weber's Law (Weber, 1850), which posits that numerical perception is based not on absolute values but rather on the ratio of the quantities being compared. Consequently, the numbers 1 through 8 are perceived as closer to each other in terms of ratio, while 16 and 24 represent a larger numerical distance both from each other and from the smaller set. This framework supports the idea that as numerical values increase, their relative distances become more pronounced, affecting how they are cognitively processed. For these reasons, we decided to classify the numbers in this manner, grounding our approach in fundamental principles of numerical cognition.

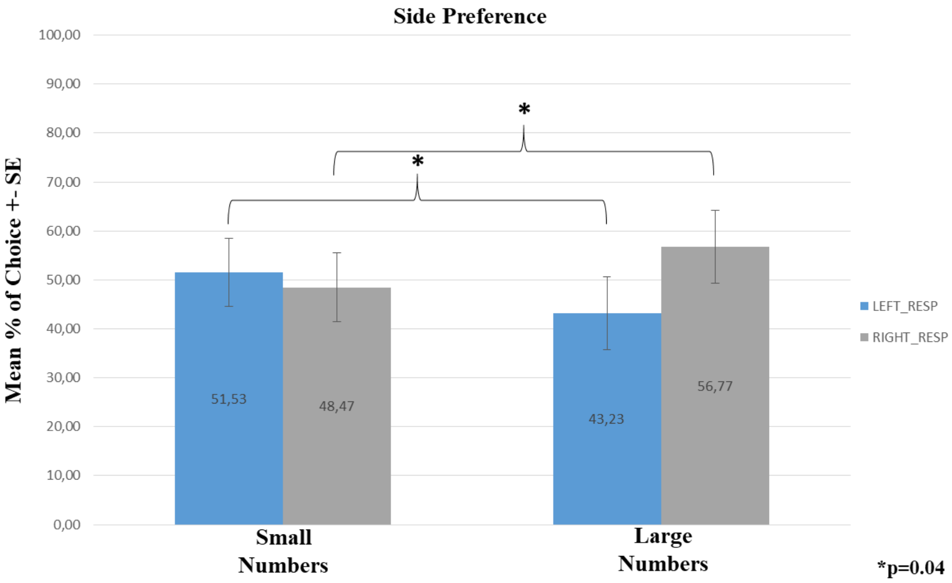
## Statistical analyses and Results

All data analyses were conducted using Jamovi (The Jamovi Project (2021); Jamovi Version 1.6 was retrieved from www.jamovi.org, accessed on 10 April 2022). All factors included in the analysis, such as the response percentages for choices made to the right and left, as well as hand bias, were subjected to an arcsine transformation. This statistical procedure was employed to stabilize the variances across the dataset and to promote a more normal distribution of the data, as the arcsine transformation often yields a more consistent representation of proportional values.

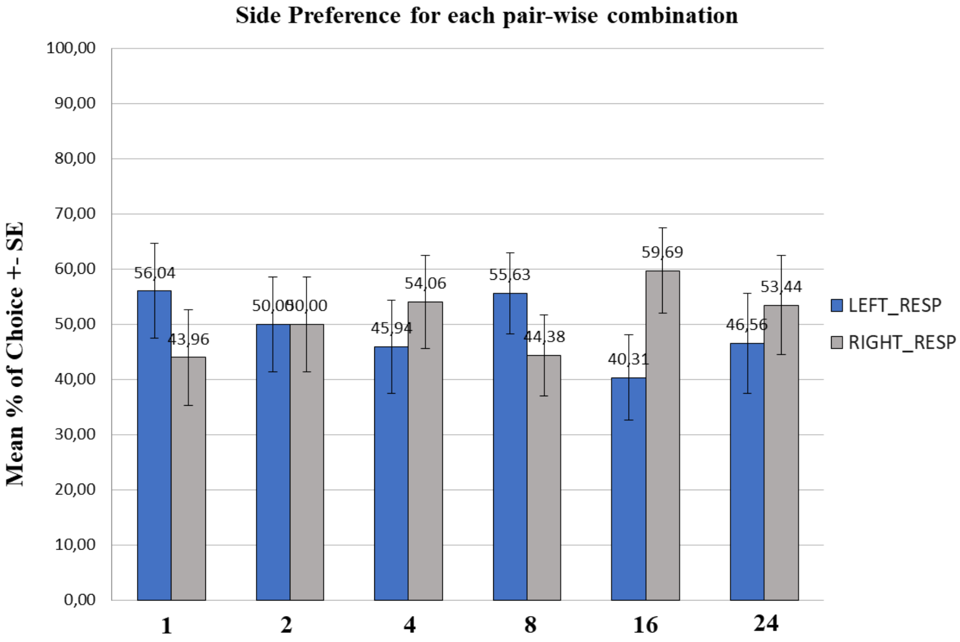
A 2x2 within-subjects repeated measures analysis of variance (ANOVA) was conducted on arcsine-transformed percentages of choices for large and small numbers. The probability value was set at $p < 0.05$. Partial eta squared ($\eta p2$) was calculated as the effect size measure, while Tukey-corrected post hoc tests were performed following the ANOVA. The within-subjects factors were Side-Choice as a two level factor (Left/Right) and Pairwise as a two level factor (*Large* including the pairs 16 and 24, while *Small* including the pairs 1, 2, 4 and 8). Results showed a significative interaction effects between the two independent variables Side-Choice and Pairwise (F=8.779; p*=0.01; ηp2=0.369). Specifically, post-hoc tests revealed that monkeys choose significantly more at left for small numbers compared to big numbers (p*=0.04) and at right for big numbers compared to small ones (p*=0.04) (see Fig 15.a).

Subsequently, we conducted a 2x6 within-subjects repeated measures analysis of variance (ANOVA) on arcsine-transformed percentages of choices for each pairwise combination. The within-subjects factors were Side-Choice as a two level factor (Left/Right) and Pairwise as a six level factor (1x1, 2x2, 4x4, 8x8, 16x16, 24x24). Results revealed no significative interaction effects between the two independent variables Side-Choice and Pairwise (F=1.419; p=0.22; ηp2=0.086) (Figure 15.b).

To determine whether the observed side preference was related to hand bias, a 2x2 within-subjects repeated measures analysis of variance (ANOVA) was conducted on arcsine-transformed percentages of hand preference for small and large numbers. The within-subjects factors were Hand-Choice as a two level factor (Left/Right) and Pairwise as a two level factor (Large including the pairs 16 and 24/Small including the pairs 1, 2, 4 and 8). Although a significant interaction was found between the two independent variables, Pairwise (Large/Small) and Hand (Left/Right) (F = 5.755, p*= 0.03, $\eta p^2 = 0.277$), suggesting a main effect of these factors, post-hoc tests did not reveal any significant differences (Fig. 16).



(a)



(b)

***Figure 15.*** *(a) Histograms representing the mean percentages of right (grey columns) and left (blue columns) responses related to the trials with small numbers (Small Numbers, i.e. 1x1, 2x2, 4x4, 8x8) and large numbers (Large numbers, i.e. 16x16, 24x24). The percentage of left-side choices is significantly higher for small numbers compared to the large numbers (*p=0.04) and the percentage of right-side choices is significantly higher for large numbers than the small ones (*p=0.04).* (b) *Histograms representing the mean percentages of the right (grey columns) and left (blue columns) side choices for each pair-wise combination (1x1, 2x2, 4x4, 8x8, 16x16, 24x24), showing any significative difference between individual pairs.*

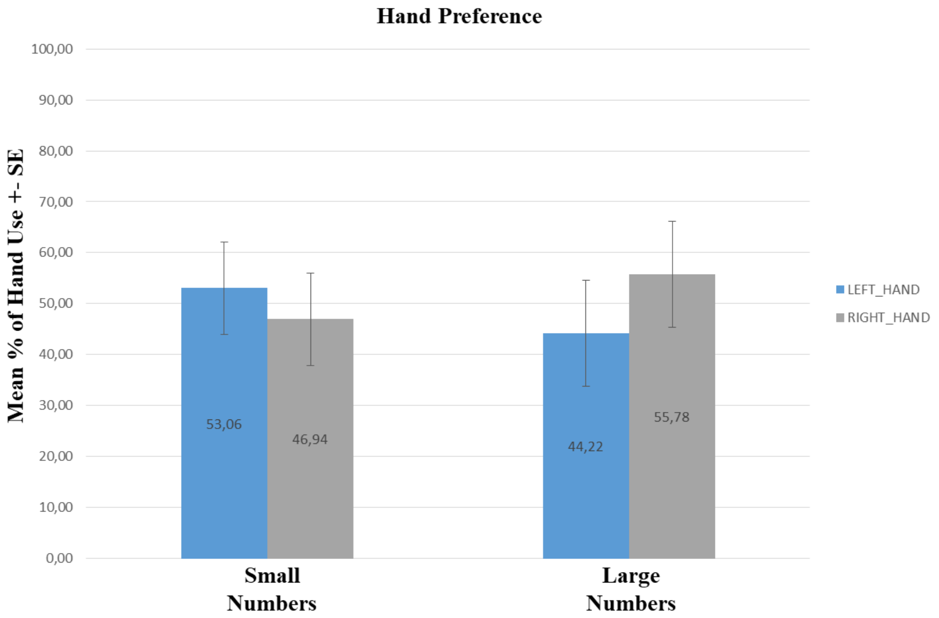


***Figure 16.*** *The histograms show the mean percentage of the right and left hand use (grey columns and blue columns respectively) related to the trials with small numbers (Small Numbers, i.e. 1x1, 2x2, 4x4, 8x8) and large numbers (Large numbers, i.e. 16x16, 24x24).*

## *Experiment II.*

### Subjects and apparatus

In the second experiment we tested 12 individuals: 2 rhesus macaques (*Macaca mulatta*) and 10 crab-eating macaque (*Macaca fascicularis*) who participated to the Experiment I. The age of the monkeys ranged from 3 to 14 years (mean age = 5.33, sd = 3.938). The subjects were tested in the same testing cages as experiment I and we employed the same food boards. All housing and procedures used in this research complied with the current guidelines in the matter of care and use of laboratory animals (European Community Council Directive No. 86–609), and were authorized by our local ethics board (03.10.18) and the French Ministry of Research (10.10.18; authorization no. 15091_2018071014483295_v2).

### Experimental Procedure

In Experiment II we used just two pair-wise combinations of food quantities: 4x4 (small quantity) and 16x16 (large quantity), which turned out to be effective in Experiment I. For each subject, the experimental session consisted of two sequences ("Sequence A - Habituation large" and "Sequence B - Habituation small") of thirty-three trials each. Both sequences were characterized by three blocks, each one consisting of a habituation phase of 10 trials with the same pairwise combination (4x4 in "habituation small" and 16x16 in "habituation large" respectively), followed by one *novel trial* with the other pairwise of food (novel phase: 16x16 for the "habituation small" and 4x4 for the "habituation large" respectively). Therefore, the sequence "Habituation large" consisted of thirty habituation trials with the pairwise combination 16x16 and three novel trials with 4x4. In contrast, the Sequence "Habituation small" involved thirty habituation trials with 4x4 pairwise combination and three novel trial with 16x16.

### Data Elaboration

For each animal, the experimental session was coded as previously explained for experiment I. A second researcher coded a random 20% of videos to assess reliability. The reliability scores between coders were very high (all $\kappa > 0.8$). Two individuals (*Macaca fascicularis*) did not complete the task due to technical issues during its execution. The two pairwise combinations of identical food quantities (4x4 and 16x16) were classified as follows: the 4x4 combination was considered a "small" quantity, while the 16x16 combination was considered a "large" quantity. Since the number of trials in the two phases—habituation and test—was unequal, we adapted our comparison method from Van Herwegen and colleagues' (2008) study on number discrimination in infants and toddlers with Williams syndrome (Van Herwegen et al., 2008). In their research, infants were first habituated to a certain numerosity and then presented with a novel numerosity. To assess whether the infants noticed a change, the researchers compared the last three trials from the habituation phase with the first trial of the test phase.

In our study, to ensure a comparable approach, we compared, for each session, the percentage of right- or left-hand/side choices in the last three habituation trials of each block—yielding a total of nine habituation trials across three blocks—with the percentage of right- or left-hand/side choices in the three novel test trials for each individual. This approach allowed us to examine whether a shift in choice behavior occurred between the final habituation phase and the presentation of the new quantity.

## Statistical analyses and Results

All data analyses were conducted using Jamovi (The Jamovi Project (2021); Jamovi Version 1.6 was retrieved from www.jamovi.org, accessed on 10 April 2022). All factors included in the analysis, such as the response percentages for choices made to the right and left, as well as hand bias, were subjected to an arcsine transformation. This statistical procedure was employed to stabilize the variances across the dataset and to promote a more normal distribution of the data, as the arcsine transformation often yields a more consistent representation of proportional values.

A 2x2x2 within-subjects repeated measures analysis of variance (ANOVA) was conducted on arcsine-transformed percentages of choices for habituation and novel trials in sequence A and B. The probability value was set at $p < 0.05$. Partial eta squared (ηp2) was calculated as the effect size measure, while Tukey-corrected post hoc tests were performed following the ANOVA. The within-subjects factors were: *sequence* as a two level factor (A – Habituation Big / B – Habituation Small); *phase* as a two level factor (Habituation trials/Novel trials); *side choice* as a two level factor (Left/Right). Results showed a significative interaction effects between the three independent variables (Sequence*Phase*SideChoice, F=13.689; p*=0.004; ηp2=0.554). Specifically, post hoc tests revealed a significantly higher percentage of left choices for "novel pair 4x4" - preceded by 16x16 habituation phase - compared with the percentage of left choices for "novel pair 16x16" (p*=0.01). Additionally, the percentage of right choices was higher for the novel pair "16x16" compared to "4x4" (p*=0.01) (Fig. 17).

A 2x2x2 within-subjects repeated measures analysis of variance (ANOVA) was conducted on arcsine-transformed percentages of hand preference for habituation and novel trials in sequence A and B. The within-subjects factors were: *sequence* as a two level factor (A – Habituation Big / B – Habituation Small); *phase* as a two level factor (Habituation trials/Novel trials); *Hand* as a two level factor (Left/Right). The results did not reveal a significant interaction between the three variables (Sequence*Hand*Phase; F=3.892; p=0.07; $\eta p^2$=0.261). However, a significant interaction was found between Sequence and Hand (F=12.138; p*=0.005; $\eta p^2$=0.525). Specifically, post-hoc tests revelead that monkeys use significantly more the left hand in the sequence A compared to the sequence B (p*=0.02) and the right hand in the sequence B compared to the sequence A (p*=0.02) (Fig. 18).

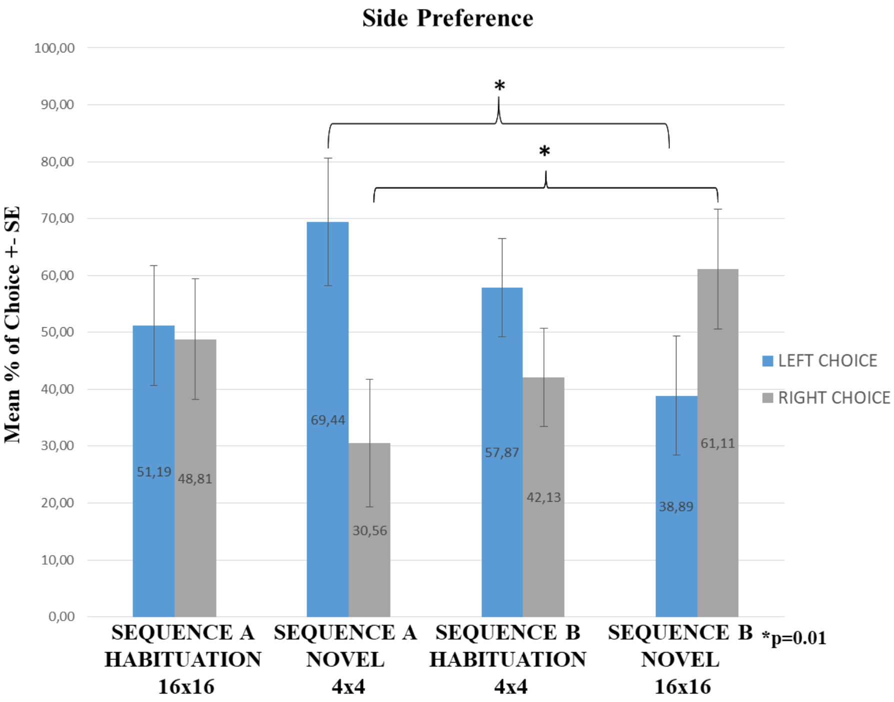


*Figure 17.* *The histograms show the mean percentage of left (blue columns) and right-side choices (grey columns) for the last three habituation trials of each block of the sequence A ("SEQUENCE A HABITUATION 16x16", i.e. trials consecutively repeated with the pair-wise combination 16x16) and the sequence B ("SEQUENCE B HABITUATION 4x4", i.e. trials consecutively repeated with the pair-wise combination 4x4) and for the three novel trials of the sequence A ("SEQUENCE A NOVEL 4x4", i.e. novel trials with the pair-wise combination 4x4) and of the sequence B ("SEQUENCE B NOVEL 16x16", i.e. novel trials with the pair-wise combination 16x16). Results report a significantly higher percentage of left-side choices for "novel pair 4x4" - preceded by 16x16 habituation phase - compared with the percentage of left-side choices for "novel pair 16x16" (p*=0.01). Additionally, the percentage of right-side choices was higher for the novel pair "16x16" compared to "4x4" (p*=0.01).*

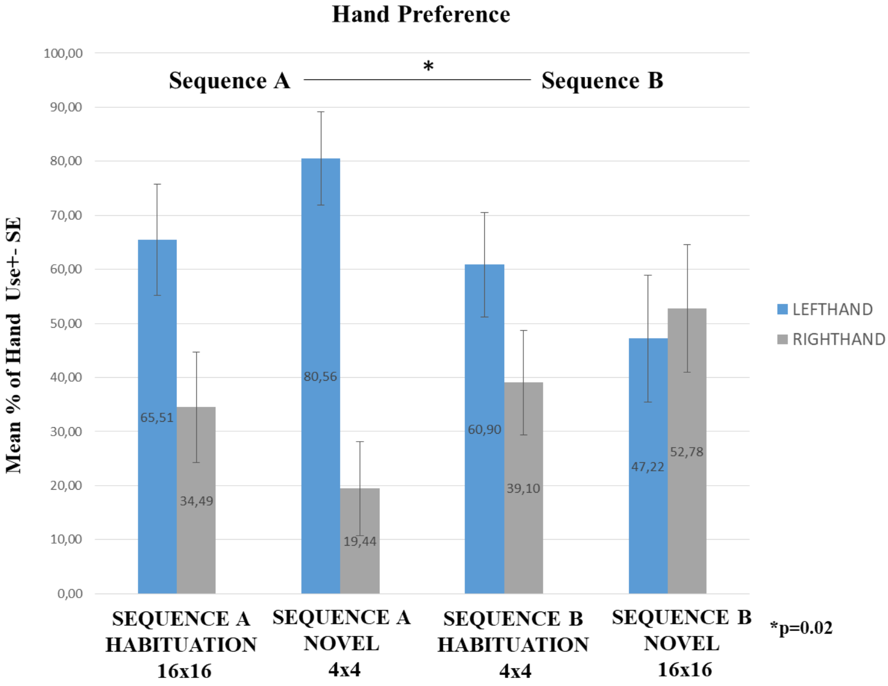


*Figure 18.* *The histograms show the mean percentage of left (blue columns) and right hand (grey columns) preference for the last three habituation trials of each block of the sequence A ("SEQUENCE A HABITUATION 16x16", i.e. trials consecutively repeated with the pair-wise combination 16x16) and the sequence B ("SEQUENCE B HABITUATION 4x4", i.e. trials consecutively repeated with the pair-wise combination 4x4) and for the three novel trials of the sequence A ("SEQUENCE A*

*NOVEL 4x4", i.e. novel trials with the pair-wise combination 4x4) and of the sequence B ("SEQUENCE B NOVEL 16x16", i.e. novel trials with the pair-wise combination 16x16). The results indicate a higher percentage (p*= 0.02) of left-hand use for sequence A compared to sequence B, regardless of the phase (Habituation or Novel), and a higher percentage (p*= 0.02) of right-hand use for sequence B compared to sequence A, also independent of the phase (Habituation or Novel).*

# **Study 2: Do emotions influence spatial bias for numbers in monkeys?**

## Abstract

According to the mental number line (MNL), humans spatially associate numbers in a left-to-right configuration, with smaller values typically represented on the left and larger values on the right. One hypothesis that seeks to explain this phenomenon is the *motivational hypothesis*. This hypothesis suggests that the spatial bias observed in the SNARC effect arises because larger numerosities, which can be considered as more desirable in appetitive contexts, activate the left hemisphere (linked to positive emotions) and result in a preference for the right hemispace. Conversely, smaller numerosities activate the right hemisphere, leading to a preference for the left hemispace. In negative or aversive contexts, however, the orientation of the SNARC effect might diminish or even reverse.

The *motivational hypothesis* has yet to be empirically demonstrated and remains a subject of ongoing investigation. To explore this phenomenon in non-human primates, we conducted three behavioral studies involving two species of macaques (*Macaca mulatta* and *Macaca fascicularis*). In the first study (Experiment III), subjects were presented with equal amounts of food on both the left and right sides (2x2 and 16x16), each associated with either a neutral or negative stimulus, which was simultaneously in view to the monkey. In the second and third studies (Experiment IV and V), subjects were presented with an equal number of stimuli (2x2 and 6x6), positioned on the left or right side on a touchscreen, and associated with either neutral or negative values. Monkeys had to touch the stimulus to complete the trial and receive a food reward. Our findings did not provide evidence that aversive stimuli can modify the Mental Number Line in macaques.

## Introduction

The mechanism underlying the SNARC effect remains unclear, though several hypotheses have been proposed. One of the most empirically testable hypothesis has been proposed by Vallortigara ("motivational hypothesis" – Vallortigara, 2018) which suggests that the lateralization of emotional and motivational processing in the brain affects the orientation of spatial-numerical associations (Vallortigara 2018). According to this proposal, shifting from large to small numerical magnitudes in appetitive contexts may trigger negative emotions, engaging the right hemisphere associated with withdrawal processes, leading to a leftward behavioral bias. Conversely, shifting from small to large numerical magnitudes may evoke more positive emotions, engaging the left hemisphere associated with approach processes, resulting in a rightward behavioral bias. Vallortigara (2018) further posits that when dealing with aversive rather than appetitive stimuli, this pattern could be reduced or even reversed: the right hemisphere might be activated by the shift from small to large numerosities of negative stimuli, and the left hemisphere by the shift from large to small numerosities of aversive stimuli, potentially leading to an inversion of the typical number-space association.

To test this hypothesis, we designed three distinct experiments utilizing an equal quantity comparison paradigm. Experiment III involved food-related stimuli, while Experiment IV and V employed digital stimuli. Specifically, Experiment III and IV featured rubber/photos of snakes as negative stimuli and fabric/photos of flowers as neutral stimuli respectively, while Experiment V incorporated photos of facial expressions, with negative and neutral valence.

Snakes were chosen as negative stimuli based on evidence that both laboratory and wild-reared macaques exhibit pronounced avoidance behaviors towards snakes or snake-like items (Etting et al., 2014; Isbell, 2006; Mineka et al., 1984; Nelson et al., 2003; Rivera et al., 2020; Shibasaki & Kawai, 2009; Weiss et al., 2015), with some individual variability observed (Nelson et al., 2003). Additionally, macaques display heightened sensitivity to snakes compared to other potentially threatening animals, such as spiders (Dinh et al., 2022; Kawai & Koda, 2016). The rubber/photos of snakes in our study were presented in a striking posture, which is known to provoke a stronger threat response (Etting & Isbell, 2014). Flowers were chosen as neutral stimuli due to their common use as non-threatening distractors in previous macaque studies (Cook & Mineka, 1989; Öhman & Mineka, 2001; Shibasaki & Kawai, 2009).

In Experiment V, the use of neutral and threat-related facial expressions was informed by previous studies demonstrating that rhesus macaques can perceive and respond to pictorial depictions of conspecifics (Parr et al., 2000; Parr & Heintz, 2009; Rosenfeld & Van Hoesen, 1979), exhibiting behaviors such as retreat, threat responses, and vocalizations (Fagot et al., 2010; Rosenfeld & Van Hoesen, 1979; Sackett, 1965). We hypothesize that macaques will display a SNARC effect in trials involving neutral stimuli, characterized by a leftward bias for small numbers and a rightward bias for large numbers. Conversely, we anticipate a reduction or potential reversal of this effect in trials with negative stimuli, reflecting the influence of emotional and motivational factors.

## *Experiment III.*

### Subjects

In the current experiment, we tested 11 adult monkeys, 2 rhesus macaques (*Macaca mulatta*) and 9 crab-eating macaques (*Macaca fascicularis*), housed at the *Institut des Sciences Cognitives Marc Jeannerod* (Lyon, France). The age of the monkeys ranged from 3 to 15 years (mean age = 5,63; sd = 4,43) (see Table II). The subjects were accommodated in the same testing cages as Experiment I and II by using the same apparatus with some modifications.

All housing and procedures used in this research complied with the current guidelines in the matter of care and use of laboratory animals (European Community Council Directive No. 86–609), and were authorized by our local ethics board (03.10.18) and the French Ministry of Research (10.10.18; authorization no. 15091_2018071014483295_v2).

| Subject | Species | Sex | Age (years) | Task |
|---|---|---|---|---|
| **Luffy** | *Macaca fascicularis* | M | 3 | Experiment III |
| **Ciro** | *Macaca fascicularis* | M | 4 | Experiment III, IV, V |
| **Genny** | *Macaca fascicularis* | M | 4 | Experiment III, IV, V |
| **Platon** | *Macaca fascicularis* | M | 5 | Experiment III |
| **Ace** | *Macaca fascicularis* | M | 3 | Experiment III |
| **Scooby** | *Macaca mulatta* | M | 15 | Experiment III |
| **Samurai** | *Macaca mulatta* | M | 14 | Experiment III |
| **Yogi** | *Macaca fascicularis* | M | 4 | Experiment III |
| **Edward** | *Macaca fascicularis* | M | 4 | Experiment III, IV, V |
| **Merry** | *Macaca fascicularis* | M | 3 | Experiment III |
| **Pippin** | *Macaca fascicularis* | M | 3 | Experiment III |
| **Tosh** | *Macaca fascicularis* | M | 14 | Experiment IV and V |
| **Diana** | *Macaca mulatta* | F | 16 | Experiment IV and V |
| **Jabba** | *Macaca fascicularis* | M | 14 | Experiment IV and V |

***Table II.*** *Table of information (Names, Species, Sex, Age) of experimental subjects in Experiment III, IV and/or V.*

## Apparatus and stimuli

The only modification to the apparatus used in Experiment I and II involved attaching a grey plastic square to the tray, centrally positioned between the two food containers. Two strips of tear-off adhesive tape were affixed to this plastic square, onto which negative or neutral stimuli were placed. The negative stimuli consisted of three different rubber snakes toys (circa 6.5 cm in height and 7 cm in width) in varying colors and forms (see Fig.19.b), while the neutral stimuli included three fabric flowers toys (circa 5.5 cm in height and 5 in width), also of different shapes and colors (see Fig.19.a). Each stimulus had two strips of adhesive tape on its underside for easy attachment and removal during trials within a session.

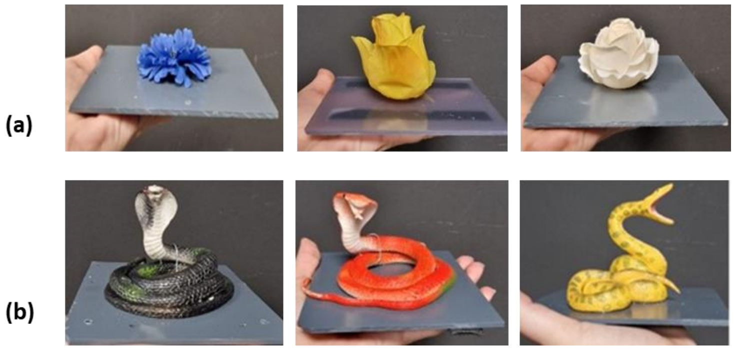


*Figure 19. Stimuli used in the Task III: (a) blue, yellow and white fabric flower as neutral stimuli and (b) black, red and yellow rubber snake as negative stimuli.*

## Experimental Procedure

In Experiment III, macaques (N=11, see Table II) were exposed to two containers filled with an equal quantity of raisins (2 pieces or 16 pieces, randomly alternating), each associated with either an aversive stimulus, represented by a rubber snake toy, or a neutral stimulus, represented by a fabric flower toy. In this experiment, given the use of only two numerical quantities without a reference number, we selected 2 and 16 as pairs to ensure a significant numerical difference. This choice minimized the potential influence of similarity-based effects, such as the distance effect, and allowed for a more precise investigation of numerical comparison. The snake and flower toys were presented in three different colors and shapes to prevent habituation. Each toy was positioned between the two trays, and the trials (aversive vs neutral) were counterbalanced. Each experimental subject underwent two sessions comprising 18 trials each, during which aversive stimuli (snake, test trials) and neutral stimuli (flower, control trials) were presented in pseudorandom order. If the monkey failed to direct its gaze towards both food containers for a duration exceeding ten seconds, the trial was aborted, and the tube racks were subsequently removed. Once the subject selected, reached for, and grasped a single raisin from one of the two (left or right) subgroups of food, the experimenter removed the food containers. Consequently, in each trial, the reward always consisted of a single piece of food, aiming to mitigate any decline in motivation during the experiment. If, after ten seconds, the monkey did not pick the food item, the experimenter removed the tube racks with food (see Experimental protocol in Fig. 20). The subjects did not undergo any training sessions but instead experienced a familiarization phase to ensure their comfort with the experimental apparatus. Additionally, each subject was tested individually and isolated from the group during the experimental procedures.

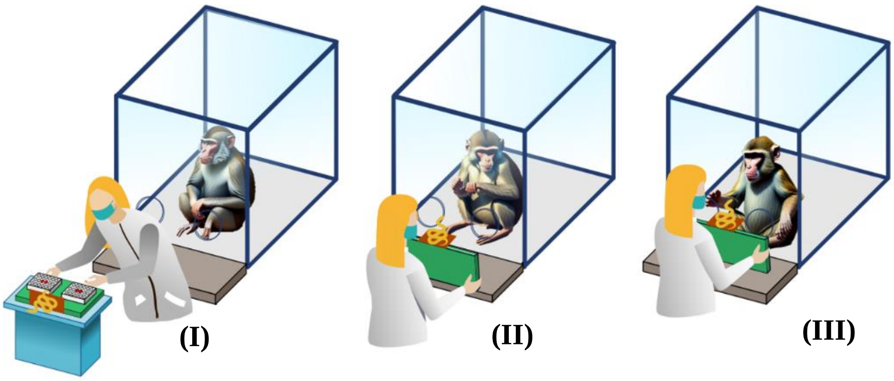


*Figure 20. Illustration of the experimental procedure: (I) The experimenter, with their back turned to the monkey, prepares food containers by placing either the negative or neutral stimulus; (II) The experimenter presents the tray just out of the monkey’s reach, waiting for the monkey to look at both containers; (III) The experimenter then places the containers within reach, allowing the monkey to choose between the left or right container and take one raisin.*

## Data elaboration

For each animal, the experimental session was coded offline using Avidemux 2.7.8 Release by the experimenter. A second researcher coded a random 20% of videos to assess reliability. The reliability scores between coders were very high (all $\kappa > 0.8$). For each experimental trial, the following variables were coded: the number of stimuli presented to the monkey (2 or 6), the choice made by the subject (right or left food container), the hand used to grasp the food and the stimulus (Neutral or Negative) used during the trial. Trials in which the monkey did not look at both food containers (left and right) were classified as "inaccurate" and were excluded from the analysis. This exclusion was essential to prevent potential biases in the results, as it was uncertain whether the monkeys had adequately attended to and assessed both food quantities in these trials.

The two possible pair-wise combinations of the same quantity of pieces of food (2x2, 16x16) were classified as follows: 2x2 was considered as a small quantity, whereas 16x6 was defined as a large quantity.

**Statistical analyses and Results**

All data analyses were conducted using Jamovi (The Jamovi Project (2021); Jamovi Version 1.6 was retrieved from www.jamovi.org, accessed on 10 April 2022). All factors included in the analysis, such as the response percentages for choices made to the right and left, as well as hand bias, were subjected to an arcsine transformation. This statistical procedure was employed to stabilize the variances across the dataset and to promote a more normal distribution of the data, as the arcsine transformation often yields a more consistent representation of proportional values.

A 2x2x2 within-subjects repeated measures analysis of variance (ANOVA) was conducted on arcsine-transformed percentages of choices for small (2x2) and large (16x16) pairs in neutral and negative trials. The probability value was set at $p < 0.05$. Partial eta squared (ηp2) was calculated as the effect size measure, while Tukey-corrected post hoc tests were performed following the ANOVA. The within-subjects factors were; *Side-Choice* as a two level factor (Left/Right), *Amount* as a two level factor (Large including the pair 16/Small including the pair 2) and *Stimulus* (Neutral including trials with flower-toy stimulus/Negative including trials with snake-toy stimulus). Results showed no significative interaction effect between the three independent variables (F=0.763; p=0.403; ηp2=0.071) (Fig. 21), suggesting that there is no side-choice bias related to the amounts in either neutral trials or those involving a negative stimulus.

A 2x2x2 within-subjects repeated measures analysis of variance (ANOVA) was conducted on arcsine-transformed percentages for the hand preference for small and large pairs in neutral and negative trials. The within-subjects factors were: *Hand-Choice* as a two level factor (Left/Right), *Amount* as a two level factor (Large including the pair 16/Small including the pair 2) and *Stimulus* (Neutral including trials with flower toy stimulus/Negative including trials with snake toy stimulus). Results showed no significative interaction effect between the three independent variables (Hand*Amount*Stimulus; F=0.366; p=0.558 ηp2=0.035) (Fig. 22), suggesting no side-hand bias associated with the amounts in either neutral trials or those involving a negative stimulus.

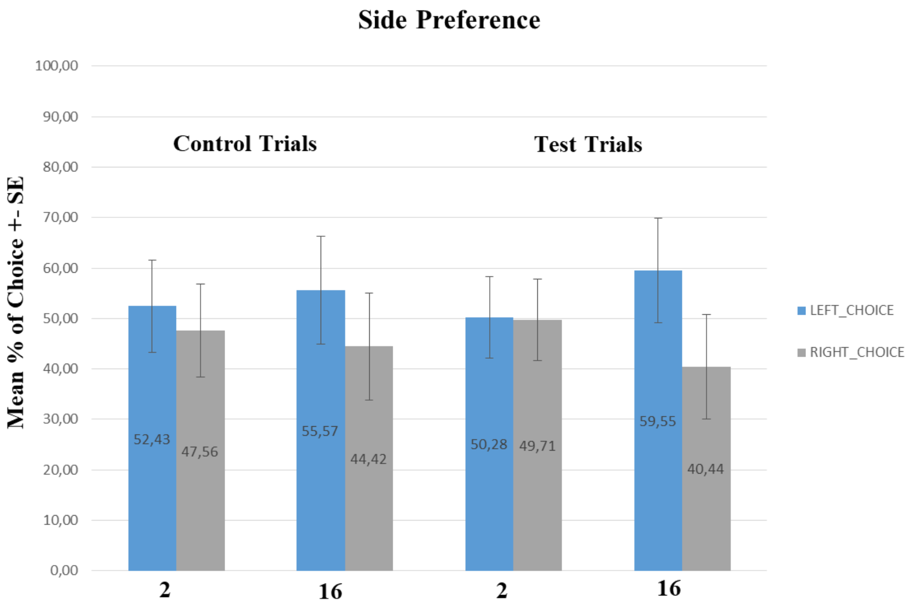


***Figure 21.*** *Histograms representing the mean percentages of right (grey columns) and left (blue columns) responses related to the quantity 2 and 16 in control trials (trials with the fabric flower) and test trials (trials with the rubber snake).*

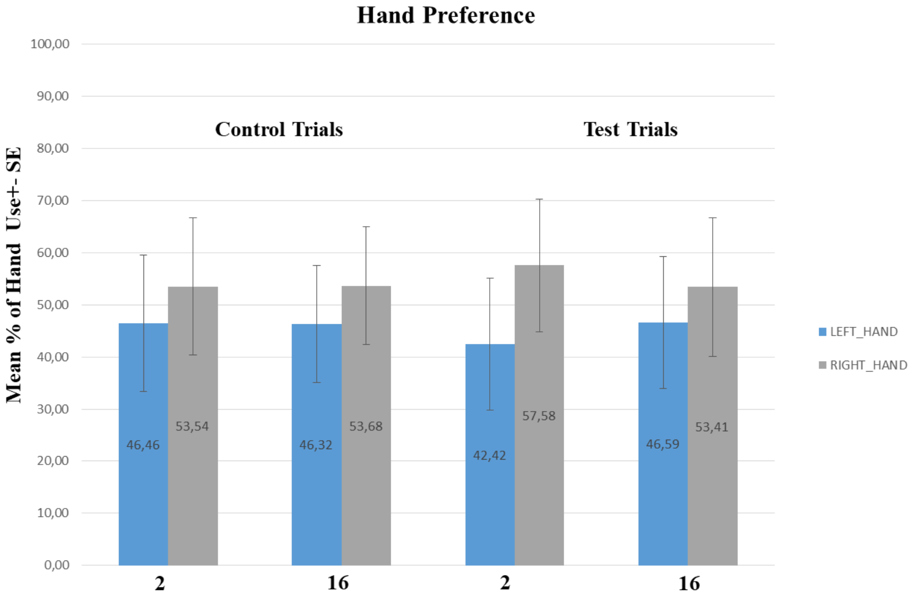


***Figure 22.*** *Histograms representing the mean percentages of right (grey columns) and left (blue columns) hand responses related to the quantity 2 and 16 in control trials (trials with fabric flower) and test trials (trials with rubber snake).*

## *Experiment IV. and V.*

### Subjects

In the current experiment, we tested 6 adult monkeys, 1 rhesus macaques (*Macaca mulatta*) and 5 crab-eating macaques (*Macaca fascicularis*), housed at the Institut des Sciences Cognitives Marc Jeannerod (Lyon, France). The age of the monkeys ranged from 4 to 16 years (mean age = 8,4; sd =6,06) (see Table II). Of these, 3 monkeys had no prior experience with touchscreens, while 3 had previous touchscreen experience.

### Apparatus and stimuli

Experiments IV and V were conducted using an Automated Learning Device for Monkeys (ALDM), which consisted of a box equipped with an LCD touchscreen monitor and a reward system. This device was mounted on a mobile trolley, allowing monkeys to interact with it freely without physical constraints. The portability of the ALDM facilitated testing within the monkeys' home cages, thereby reducing the stress typically associated with relocation to a testing environment. A GoPro camera (model: GoPro Hero 7) was installed to record the sessions, enabling offline coding and analysis.

Both experiments were programmed using PsychoPy 3 - v2021.1.1, utilizing a Python script. The stimuli consisted of four different shape-color photos of snakes (test trials) and flowers (control trials) in quantities of two and six for Experiment IV (see Figure 23.a-b for images of all stimuli), and neutral facial expressions (control trials) and threatening expressions (test trials) for Experiment V (see Figure 24). All stimuli were presented at a resolution of 561x433 pixels.

*Figure. 23 (a) Negative and (b) Neutral stimuli used in Experiment IV*

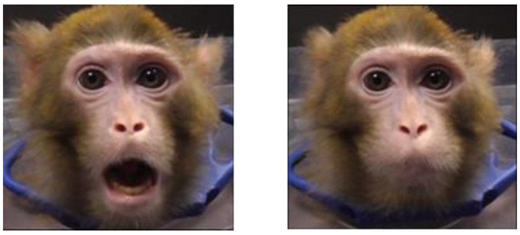


*Figure 24. Threatening (Left) and Neutral (Right) facial expression used as negative and neutral stimuli in Experiment V*

## Experimental Procedure

The experimenter transported the ALDM system to the monkey's home cage, positioned it, and then left the monkey alone with it. The session began when the isolated subject touched the triangle at the center of the screen. Following this, two rectangles appeared on the left and right sides of the screen, each representing the same numerical magnitude. When the monkey selected and touched one of the two rectangles, a juice reward was dispensed, regardless of the choice (see Fig. 25). Each subject underwent two sessions of 80 trials (40 control trials and 40 test trials in a pseudorandom order).

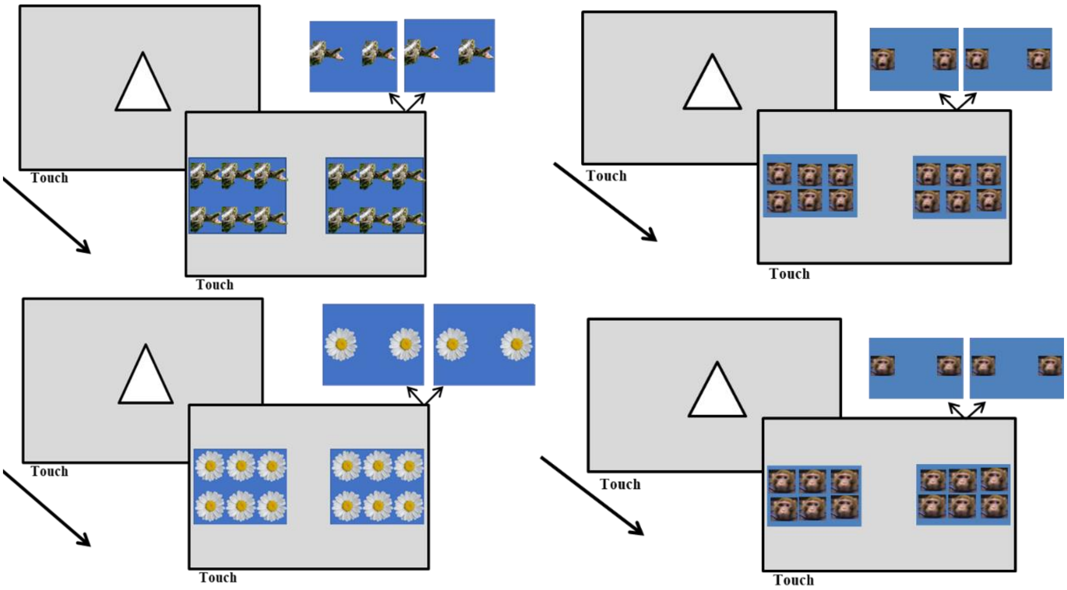


*Figure 25. Illustration of the Experimental Procedure used in Expriment IV (Left) and Experiment V (Right). The monkey first touched the center triangle, after which two rectangles containing 2 or 6 stimuli appeared on both sides. The monkey then touched one of the two stimulus sets and received a juice reward regardless of its choice. This procedure was repeated across 80 trials, with negative and neutral stimuli, in quantities of two or six, presented in a pseudorandom order.*

## Data elaboration

For each animal, the experimental session was coded offline using Avidemux 2.7.8 Release by the experimenter. For each experimental trial, the following variables were coded: the number (2 or 6) and the *valence* (positive or negative) of the stimuli presented to the monkey, the choice made by the subject (right or left rectangle), the hand used to interact with one of the stimuli, the confidence level of the reaction time and the overall quality of the trial. Regarding the confidence level of the reaction time, we classified the reaction time as invalid in trials where the monkey did not direct its attention towards the stimulus at the moment it appeared. Specifically, the validity of the reaction time was determined by whether the monkey was looking at the stimulus when it first became visible. If the monkey's gaze was not aligned with the stimulus at its onset, the reaction time for that trial was excluded from the analysis. Regarding the overall quality of the trial, a rating of 0 was assigned if the monkey touched the stimulus without visually attending to it or executed a repetitive swiping motion. A rating of 1 was given if the monkey initiated the hand movement before stimulus presentation, had its hand already positioned on the screen, or appeared not to pay attention to the stimuli. Trials rated as 0 or 1 in terms of quality were excluded from the analysis. This exclusion was necessary to avoid potential biases in the results, as it was unclear whether the monkeys had properly attended to the stimuli or had made their choice prior to fully evaluating them. One monkey was excluded from the analysis due to highly stereotyped behavior.

## Statistical analyses and Results

All data analyses were conducted using Jamovi (The Jamovi Project (2021); Jamovi Version 1.6 was retrieved from www.jamovi.org, accessed on 10 April 2022). All factors included in the analysis, such as the response percentages for choices made to the right and left, as well as hand bias, were subjected to an arcsine transformation. This statistical procedure was employed to stabilize the variances across the dataset and to promote a more normal distribution of the data, as the arcsine transformation often yields a more consistent representation of proportional values.

**Experiment IV**. A 2x2x2 within-subjects repeated measures analysis of variance (ANOVA) was conducted on arcsine-transformed percentages of choices for small and large pairs in neutral and negative trials. The probability value was set at $p < 0.05$. Partial eta squared (ηp2) was calculated as the effect size measure. The within-subjects factors were: *Side-Choice* as a two level factor (Left/Right), *Amount* as a two level factor (Large including the pair 6/Small including the pair 2) and *Stimulus* (Neutral including trials with flower images/Negative including trials with snakes images). Results showed no significative interaction effect between the three independent variables (F=0.325; p=0.599; ηp2=0.075) (Fig. 26).

Similarly, A 2x2x2 within-subjects repeated measures analysis of variance (ANOVA) was conducted on arcsine-transformed percentages of hand preference for small and large pairs in neutral and negative trials. The within-subjects factors were: *Hand-Choice* as a two level factor (Left/Right), *Amount* as a two level factor (Large including the pair 6/Small including the pair 2) and *Stimulus* (Neutral including trials with flower images/Negative including trials with snakes images). The results did not indicate a significant interaction effect between the three independent variables *Hand*, *Amount*, and *Stimulus* ($F = 1.192$; $p = 0.336$; $\eta p^2 = 0.230$) (Fig. X). However, a significant interaction was initially observed between the two variables *Hand* and *Amount* ($F = 12.461$; $p^* = 0.02$; $\eta p^2 = 0.757$). Despite this, post-hoc tests failed to confirm any significant pairwise interactions between *Hand* and *Amount* (Fig. 27).

**Experiment V.** A 2x2x2 within-subjects repeated measures analysis of variance (ANOVA) was conducted on arcsine-transformed percentages of choices for small and large pairs in neutral and negative trials. The probability value was set at $p < 0.05$. Partial eta squared (ηp2) was calculated as the effect size measure. The within-subjects factors were: *Side-Choice* as a two level factor (Left/Right), *Amount* as a two level factor (Large including the pair 6/Small including the pair 2) and *Stimulus* (Neutral including trials with neutral faces images/Negative including trials with threathening faces images). Results showed no significative interaction effect between the three independent variables (F=0.032; p=0.866; ηp2=0.008) (Fig. 28).

Similarly, A 2x2x2 within-subjects repeated measures analysis of variance (ANOVA) was conducted on arcsine-transformed percentages of hand preference for small and large pairs in neutral and negative trials. The within-subjects factors were: *Hand-Choice* as a two level factor (Left/Right), *Amount* as a two level factor (Large including the pair 6/Small including the pair 2) and *Stimulus* (Neutral including trials with neutral faces images/Negative including trials with threathening faces images). Results showed no significative interaction effect between the three independent variables (F=0.019; p=0.897; ηp2=0.005) (Fig. 29).

Reaction times were excluded from the analyses because, in many trials, the subjects did not immediately direct their attention to the stimulus when it appeared. This frequent lack of alignment resulted in insufficient reliable data for meaningful analysis. Consequently, reaction times were not considered in the final evaluation.

In conclusion, neither Experiment IV nor Experiment V provided evidence of a choice or hand-use bias related to the amounts in either neutral or negative trials.

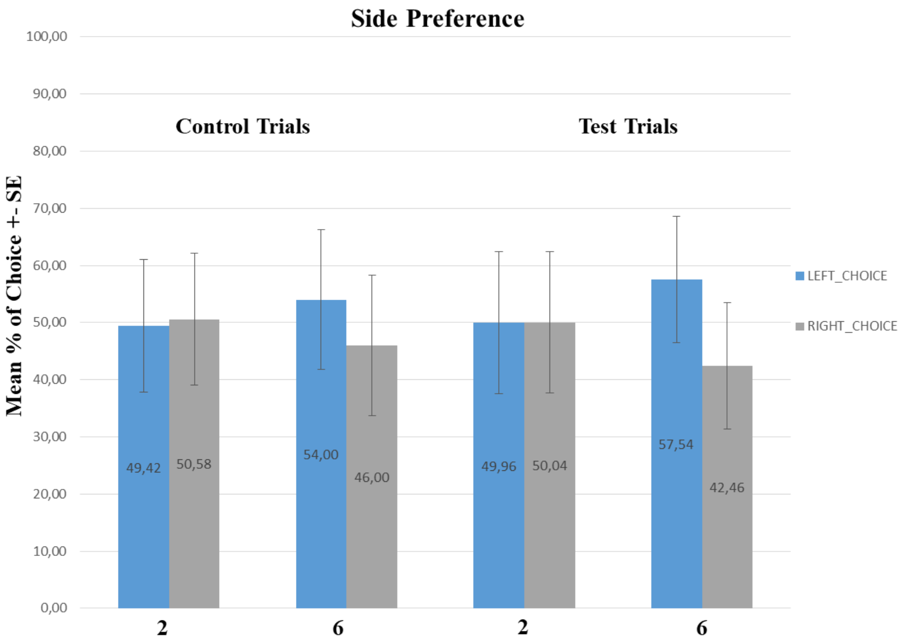


*__Figure 26.__ Histograms representing the mean percentages of right (grey columns) and left (blue columns) responses related to the quantity 2 and 6 in control trials (trials with flower stimuli) and test trials (trials with snake stimuli) in Experiment IV.*

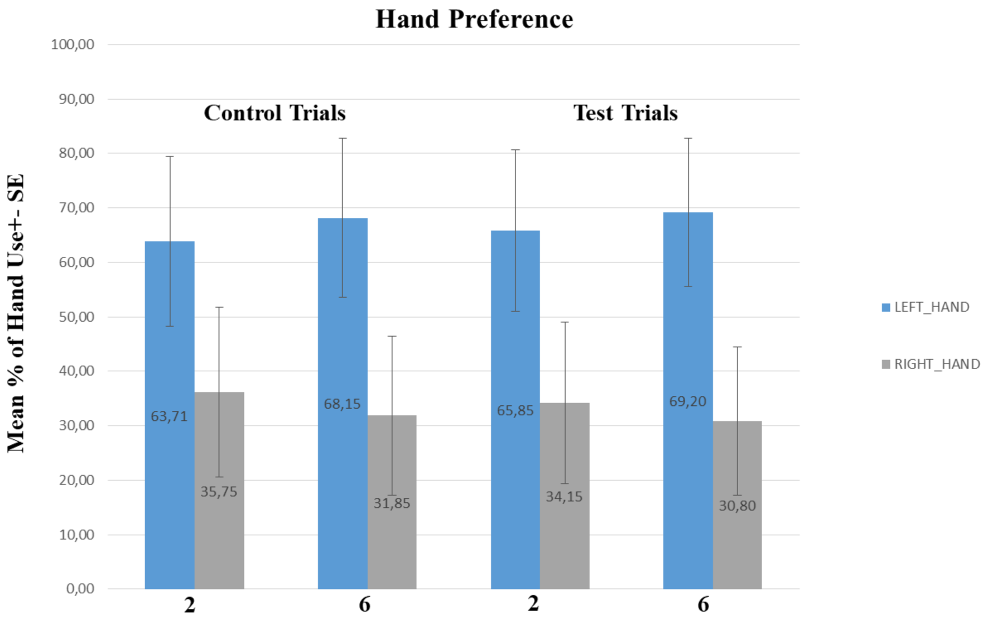


***Figure 27.*** *Histograms representing the mean percentages of right (grey columns) and left (blue columns) hand responses related to the quantity 2 and 6 in control trials (trials with flower stimuli) and test trials (trials with snake stimuli) in Experiment IV.*

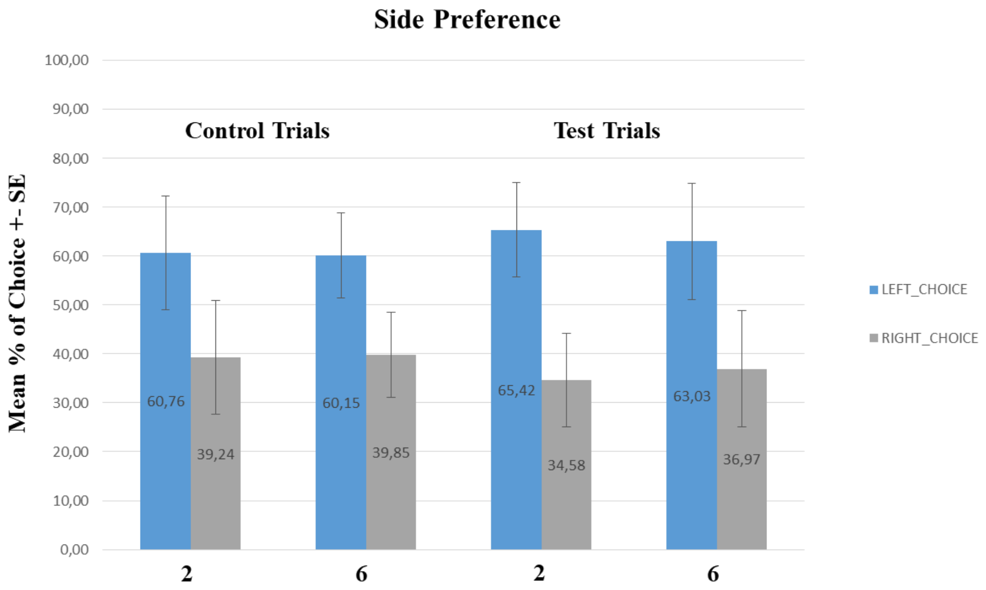


***Figure 28.*** *Histograms representing the mean percentages of right (grey columns) and left (blue columns) responses related to the quantity 2 and 6 in control trials (trials with neutral face stimuli) and test trials (trials with threat face stimuli) in Experiment V.*

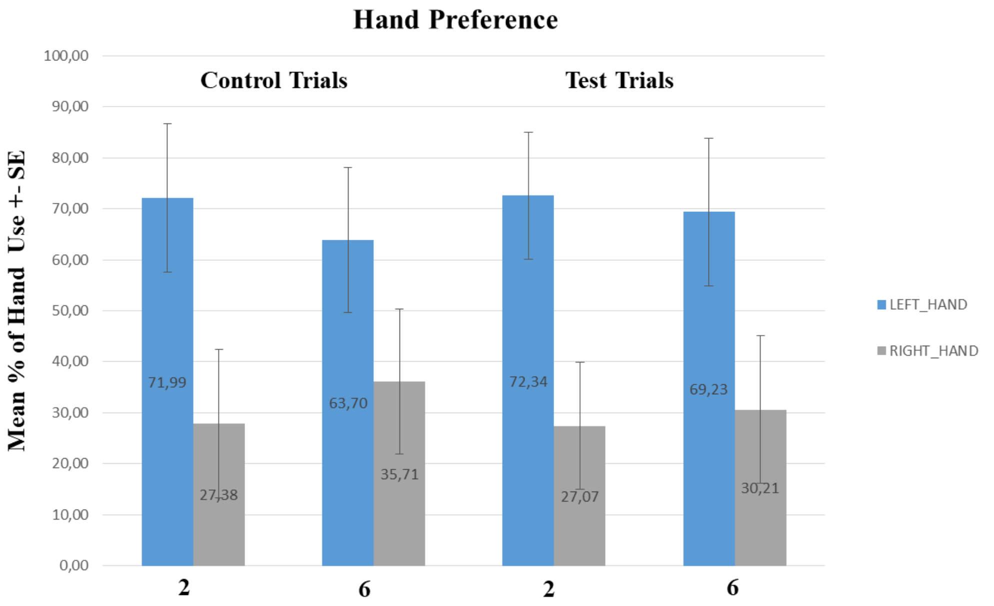


***Figure 29.*** *Histograms representing the mean percentages of right (grey columns) and left (blue columns) hand responses related to the quantity 2 and 6 in control trials (trials with neutral face stimuli) and test trials (trials with threat face stimuli) in Experiment V.*

# General Discussion

The present study aimed to assess the occurrence of a left-to-right Mental Number Line in two species of macaques using two spontaneous food-related tasks, contributing further insights into the possible biological origins of the MNL. In Experiment I, we investigated whether macaques exhibit an oriented mental number line (MNL) in a spontaneous task by presenting them with two equal options within a numerical range, but without a reference number. In contrast, Experiment II explored the emergence of the MNL when a shift in numerosity occurs after an habituation period with a specific target number, requiring the macaques to rely on comparative rather than independent numerical evaluation.

We found evidence of an oriented left-to-right MNL in both task, providing significant scientific insights that build upon the previous findings by Drucker and Brannon (2014) and Rugani et al. (2024). Specifically, in Experiment I, we observed evidence of a left-to-right mapping of numbers even in the absence of a target number. Although no significant preference emerged for individual numerical pairs, a pattern was evident when numbers were grouped into small and large categories. It's important to note that in Experiment I, the numbers presented varied within a range from 1 to 24, meaning that monkeys might have been able to perceive these numbers relatively within this range. This could explain the emergence of a general left-to-right mapping when considering the numerical values in relation to each other.

However, the absence of a consistent left-to-right MNL for individual pairs in Experiment I, compared with the more pronounced MNL in Experiment II— where an oriented mapping was observed for only two numerical pairs—suggests that being primed with a reference number and then experiencing a shift to a larger or smaller number may be key in the emergence of the SNARC effect. This implies that the relative perception of numerical values, rather than their absolute magnitude, might play a more significant role in eliciting spatial-numerical associations.

The design of the Experiment II, with some modifications, was previously used in other studies on non-human animal and human species - chicks (Rugani et al., 2015b), honeybees (Giurfa et al., 2022) and newborns (Di Giorgio et al., 2019)- yielding results consistent with the mental number line. Our results align with previous findings but contrast with those of a recent study on macaques that did not find the same outcomes in a computer-based task using a similar equal quantity comparison design (Beran et al., 2020). In their study, macaques were first habituated to a reference numerosity and then presented with either a smaller or a larger numerosity on the left and right side of the screen. However, no consistent side preference was observed. Instead, they found only limited evidence of a SNARC effect in both directions (left-to-right and right-to-left) when the two arrays differed in numerosity, suggesting a certain spatial representation of magnitude (Beran et al., 2020). We propose that our differing results may be due to the different sample size (only five rhesus macaques in Beran’s study) and the fact that our monkeys were naïve to numerical tasks, unlike those in Beran's study. Consequently, our monkeys’ responses might have been more spontaneous and automatic, less influenced by cognitive control mechanisms that could modify their natural tendencies, such as spatial-numerical representations.

A key factor that may explain the difference in results between our study and that of Beran and colleagues could be the use of ecological stimuli, such as real food, rather than digital ones. While previous studies using touchscreen tasks have demonstrated spatial biases in primates, these studies employed different paradigms. For instance, Adachi (2014) trained chimpanzees to touch Arabic numbers from 1 to 9 in ascending order, with the numbers randomly positioned on a screen. In a later phase, only two numbers (1 and 9) were presented horizontally at the left and right of the screen. Chimpanzees responded faster when the number 1 was on the left and 9 on the right, suggesting a left-to-right spatial mapping. Similarly, Gazes and colleagues (2017) found evidence of a SNARC effect in gorillas and orangutans using a quantity discrimination task on a touchscreen computer, although there were individual variations in the direction of the bias. Additionally, Rugani et al. (2024) demonstrated that macaques exhibited better recall for targets positioned on the left when presented in smaller arrays, while they showed enhanced recall for targets on the right when presented in larger arrays.

However, the paradigms used in these studies required primates to follow specific rules, such as selecting numbers in a sequence or choosing the smaller or larger numerosity, which differs significantly from the paradigm used in both our study and Beran et al's (2020). In the latter approach, the monkeys had no rule to follow and were rewarded for any choice they made, regardless of its correctness. This constant reward structure may have led to reduced attention to the stimuli, with monkeys potentially making choices without fully evaluating the options, or simply to obtain the reward. This lack of focus could lead to more distracted and random decision-making.

In contrast, we propose that using real food as a stimulus, rather than digital representations, could increase motivation and attentional focus, potentially reducing distraction. The presence of food may make numerical stimuli more relevant to the monkeys, as suggested by Addessi (2014). Additionally, in our study, the experimenter was able to monitor whether the monkey was actively attending to the stimuli, a factor that was not controlled in Beran et al's (2020) study. This factor was important to ensure that the monkey had seen and evaluated both options before making a choice.

Additionally, Beran and colleagues employed different amounts of food and a different presentation modality (20 trials of habituation and 10 test trials versus 30 trials of habituation and 3 test trial in our task), with the habituation stimulus presented centrally. In our task, choices in the habituation phase were presented on either side, allowing us to compare responses during habituation with those during test trials. As suggested by Moro and collegues (2018), the SNARC effect may arise from various factors, including spatial representation mechanisms and different processing modes. Indeed, varying presentation modalities and contextual factors can influence whether or not the SNARC effect can be detected. Finally, the differing results may also stem from intra-population variability, a feature promoted in biological systems by natural selection to adapt to environmental challenges and changes.

Our results also reveal an absence of a clear hand preference, indicating that the observed choices are not driven by strong hand lateralization. Specifically, in Experiment I, although the use of the hand appears to correspond with the direction of the choice, spatial mapping does not appear to be significantly affected by lateralization at the level of the hand. In contrast, the results of the second experiment suggest that the monkeys preferentially employed their left hand in sequence A and their right hand in sequence B. However, this differentiation is solely related to the sequence and does not pertain to the specific numerical quantities presented (habituation or novel phase). This supports the idea that the spatial arrangement of numbers reflects a genuine spatial organization rather than being influenced by hand preference and a lateralization in hand functions. This finding is consistent with Drucker and Brannon's (2014) research on macaques, which noted a side-bias but no significant hand-bias in a task where monkeys touched the fourth dot from either the left or right on a horizontal line of dots. This is in line also with findings on humans, such as Dehaene and colleagues (1993), who found that participants responded more quickly to the left for small numbers, even when using their right hand, and to the right for large numbers, even when using their left hand (Dehaene et al., 1993). This suggests that the spatial-numerical association is governed by the spatial organization of numbers rather than hand preference. Additionally, at the population level, macaques generally do not exhibit a pronounced hand preference (McGrew & Marchant, 1997; Papademetriou et al., 2005).

An unresolved issue concerns the mechanism underlying the Mental Number Line (MNL) phenomenon. To address this, we chose to examine the motivational hypothesis, which is particularly amenable to behavioral studies, through three distinct experiments. Our results from Experiments III, IV, and V suggest that, with the use of neutral and negative stimuli, there is no spatial bias either from left to right in neutral trials or from right to left in negative trials.

Several factors may account for these results. In Experiment III, positioning a neutral or negative stimulus centrally, without varying its numerosity, likely prevented these stimuli from acquiring significant emotional value related to their magnitude. This setup may have elicited an initial reaction that was independent of numerical quantity. Furthermore, because the stimuli were food items, which typically evoke positive responses, the negative impact of the snake might have been mitigated by the food's positive association, thereby neutralizing any potential bias. This observation aligns with previous findings that monkeys can reduce their fear of snakes when these threats are associated with food (Rush et al., 1983) and that such fear responses can rapidly habituate (Nelson et al., 2003). Additionally, the response to snakes in the wild is variable and depends on factors such as the snake's proximity and movement. The use of stationary toy snakes in our study may have further diminished the perceived threat. Finally, the absence of a Mental Number Line (MNL) in neutral trials could be attributed to the lack of a reference number and/or the potential influence of negative stimuli on these trials, which may have hindered the emergence of a clear SNARC effect.

In Experiment IV and V, several factors may explain the observed results. Firstly, it is uncertain whether non-human animals can abstractly represent numerical quantities for all types of stimuli, such as images, which might mean that subjects did not perceive these stimuli as numerical values (Izard et al., 2019). Secondly, the monkeys might have quickly habituated to the limited range of emotional stimuli used—four negative and neutral stimuli in Experiment IV and one in Experiment V. Additionally, unlike Experiment I, II and III, we could not confirm that the monkeys had viewed both stimuli before making a choice, which could have affected the results, even though we excluded trials where stimuli were missed or ignored.

Additionally, the use of facial expressions in Experiment V may have been influenced by the right hemisphere's dominance in face processing (Hauser, 1993). This dominance could account for the observed preference for left-sided choices, independent of numerical magnitude. Future research should consider incorporating positive stimuli or a reference number to more effectively examine how numerical quantity shifts in response to emotionally charged stimuli. Analyzing reaction times could also provide valuable insights, though this was not feasible in the current study due to limited data.

In conclusion, our data suggest the presence of a left-to-right representation of numbers in macaques, but we lack evidence for the mechanism underlying this phenomenon, althought the widespread occurrence of Number-Space Associations in birds, honeybees, and monkeys prompts consideration of a potential shared neural mechanisms.

Future research could explore whether hemispheric lateralization in motivational processing (Vallortigara, 2018) influences the number-space association in monkeys and whether this mechanism is consistent across different non-human species. Such studies would provide deeper insights into the evolutionary origins and variability of this phenomenon across species.

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# Chapter IV. *Side Project: Investigating the effects of early social deprivation in peer and mother-reared macaques*

## Introduction

Early life experiences significantly influence the development of the neural circuits that support cognitive, emotional, and perceptual functions, with particular emphasis on experiences acquired during "critical periods" (Lenneberg, 1967; Penfield and Roberts, 1959) These periods represent critical windows during which neural activity exerts the most significant influence on the development and performance of specific behaviors. The concept of "imprinting" in social animals further underscores the significance of these periods, as it involves rapid learning that establishes behavioral patterns, such as species recognition and attachment (Immelmann, 1972; Lorenz, 1935; Marr & Lilliston, 1969)

Research on early social deprivation in monkeys further highlights the importance of critical periods in brain development. Inspired by earlier findings on brain development and imprinting, Harry Harlow and his team at the University of Wisconsin conducted groundbreaking studies in the 1950s (Harlow, 1959) on infant 'attachment (i.e. the emotional bond that infants develop with their mother, Bowlby, 1969). Shortly after birth, they removed rhesus macaque infants from their biological mothers and reared them without the presence of a human caregiver. In a famous experiment, infant monkeys were housed with two artificial "maternal" substitutes: one constructed from wire and wood, and the other wrapped in foam rubber and soft cloth.

The infants were allocated to one of the two scenarios: in one, the wire mother provided milk, while the cloth mother did not; in the other, the cloth mother provided milk, and the wire mother did not (Fig.1). Harlow discovered that the infant monkeys consistently preferred to remain with the cloth-covered mother for longer periods, regardless of which surrogate provided food. If food was only offered by the wire mother, the infants would consume it swiftly and then go back to the cloth surrogate. (Harlow & Zimmermann, 1959).

***Figure 1**. Infant rhesus macaque with cloth and wire mother surrogates (Figure from Harlow & Zimmermann, 1959).*

Harlow also conducted a comparison between infants who had been deprived of social interaction and isolated from others, and those who had not experienced social isolation

(Harlow & Zimmermann, 1959), and noted that while the non-isolated monkeys developed into healthy adults, those raised in isolation with an artificial mother exhibited atypical behaviors. This included behaviors such as increased aggression, fear, and motor stereotypies. Harlow's studies were pioneering in showing that maternal care is critical not only for physical health but also for emotional well-being, highlighting the importance of early social interactions for healthy behavioral development. These investigations offered the initial empirical support for the fundamental significance of the parent-child bond in the psychological growth of infants.

In humans, the significance of early social experience was first explored by the psychiatrist René Spitz in 1945. Spitz studied infants and children in orphanages and discovered that, despite receiving sufficient nutrition and medical care, children who lacked emotional connection and physical contact failed to thrive and, in some cases, even died (Spitz, 1945). This work highlighted the essential role of early attachment and social interaction in human development.

Subsequently, British psychologist John Bowlby formulated a theory of attachment that synthesized empirical evidence from both human and animal studies. According to Bowlby, attachment encompasses the emotional mechanisms through which a caregiver manages their child's levels of arousal (Bowlby, 1958) and is marked by a profound, lasting connection that links individuals over time and distance (Ainsworth, 1979; Bowlby, 1969). His attachment theory asserts that infants possess an innate need to yearn for closeness to their caregiver in times of stress or danger, anticipating safety and emotional reassurance support in return.

In collaboration with James Robertson in the 1930s, Bowlby noted that children exhibited significant distress when removed from their mothers, and this emotional turmoil was not alleviated by the care provided by other caregivers (Bowlby et al., 1952). Bowlby suggested that infants form bonds with their primary caregivers during the crucial window of 0 to 30 months, a developmental phase that influences their future interpersonal relationships and is referred to as "internal working models." These initial bonds play a vital role in a person's emotional and social growth, potentially influencing their relationships in adulthood for years to come.

In recent decades, there has been a growing emphasis in research on how early social experiences shape outcomes, particularly concerning the impact of early social adversity (ESA), which describes situations in which infants do not receive the social inputs expected in the early environment (e.g. due to parental separation or mental illness, abuse, or neglect) on a range of neural and psychological effects (see Table I). Studies have shown that ESA in humans is associated with worse academic and work performance, and social competence (Ford et al., 2011; Oeri & Roebers, 2022; Sansone et al., 2012; Zielinski, 2009), and a greater likelihood of developing psychiatric and substance use disorders (Dube et al., 2003; Gershon et al., 2013; Humphreys et al., 2020; Zeanah et al., 2009). One of the most consistent findings in the literature is that early social deprivation results in self-regulation difficulties, including attentional and affect regulation problems (Bridgett et al., 2015; Kennedy et al., 2017) thought to be related to atypical development in executive function. Executive function refers to a set of higher-order cognitive processes that regulate goal-directed behavior by coordinating thoughts, actions, and emotions, including attentional and inhibitory control, working memory, and cognitive flexibility.

There is a well-established link between early institutionalization and increased risk for clinical conditions characterized by executive function deficits, such as of attention-deficit/hyperactivity disorder (ADHD), conduct problems, and mood disorders (Kreppner et al., 2001; Loman et al., 2013; Pollak et al., 2010; Roy et al., 2004; Rutter et al., 2001; Sheridan et al., 2010; Wiik et al., 2011). This type of ESA is comparable to research involving separation from caregivers in animal models. Notably, an expanding body of research indicates that these negative outcomes associated with institutionalization arise via atypical development in brain networks linked to self-regulation (Holz et al., 2023; McLaughlin et al., 2014). However, many questions still exist regarding the precise mechanisms through which such brain alterations link psychological and behavioral development throughout various stages of development, including executive function specifically.

In the animal literature, much research has used macaques models to explore the consequences of early psychosocial deprivation on behavioral development in a more controlled manner than can be done with humans. In these investigations, macaques have been placed in environments that entailed substantial caregiver unavailability, which included lengthy periods of maternal separation and upbringing by human caretakers in peer-based nurseries (Balint et al., 2021; Kim & Kret, 2022; Schumann et al., 2019). Findings from these studies indicate that early psychosocial deprivation in macaques adversely affects various developmental domains, reflecting similar outcomes observed in humans. Deprived macaques show heightened responsiveness to threats, increased anxiety, aggression, motor dysfunction and compromised social relationships (Balint et al., 2021; Kim, 2022; Schumann et al., 2019). Although the majority of studies have concentrated on the earlier phases of macaque development, limited research addressing the later juvenile stage and adulthood indicates that the impacts of early deprivation can have enduring effects has been conducted (Corcoran et al., 2012; Dettmer & Suomi, 2014; Zhang et al., 2016). Indeed, Cocoran and collegues (Cocoran et al., 2012) found that 10-years old peer-reared macaques showed inhibited behavior, characterized by lower levels of locomotion and exploration compared to mother-reared ones. Similarly, Zangh and collegues (2016) found that early adversity in adolescent male rhesus monkeys was associated with the emergence of depression-like behaviors when they were under conditions of persistent mild stress (Zhang et al., 2016).

A limited number of studies have focused on the effects of early psychosocial deprivation on brain development in macaques. Wang et al. (2018) discovered that macaques deprived of maternal care around the age of three exhibit both structural and white matter abnormalities. Notably, there was a reduction in gray matter volume (GMV) in the primary visual cortex (V1), which is likely associated with impairments in visuospatial attention processing. Additionally, the researchers observed elevated fractional anisotropy (FA) values in the white matter of the left posterior superior temporal sulcus (pSTS), potentially linked to heightened anxiety and stress in these animals. This, in turn, may contribute to deficits in social cognition, particularly in their ability to infer the mental states of others (theory of mind) (Wang et al., 2018). Additionally, macaques that experience maternal deprivation display significant alterations in dendritic arborization and synaptic functioning within key brain regions, including the prefrontal cortex (PFC), hippocampus, and amygdala (Howell et al., 2014; Meaney et al., 1996; Spinelli et al., 2009). These areas are invloved in fear processing and the regulation of the hypothalamic-pituitary-adrenal (HPA) axis (Kalin et al., 2007; Mckeon et al., 2022; Raper et al., 2013). Moreover, EEG research indicates that early maternal deprivation is linked to atypical cortical activity in brain regions responsible for attention and sensorimotor processing in relation to social cues (Rayson et al., 2021; Vanderwert et al., 2015).

Recently, Rayson and collegues (2021) conducted a study on two groups of macaques (peer and mother reared), showing that pre-adolescent peer-reared macaques show atypical attention biases to emotional stimuli, which predicted greater levels of anxiety (Rayson et al., 2021). The same researchers, with the same macaque sample, also found that across adolescence into early adulthood, peer-reared animals had worse executive function skills, involving both inhibitory control and working memory deficits (Massera et al., 2023).

To examine the potential interaction between cognitive and emotional aspects of self-regulation in predicting longer-terms poor outcomes such as anxiety or ADHD-like symptoms in macaques subjected to early psychosocial deprivation, the current research involved performing behavioral observations on the same colony previously investigated by Rayson (2021) and Massera et al. (2023) at a new time point (mature adulthood: 7 years). This cohort is unique in Europe, offering a valuable opportunity to examine the effects of early social deprivation under highly controlled conditions.

We hypothesized that peer-reared macaques would continue to exhibit more anxiety-related and executive function difficulties compared to those reared by their mothers. Preliminary results from this investigation are presented here in this thesis.

| *Project* | *Group (compared to control)* | **Outcomes** | | | |
|---|---|---|---|---|---|
| | | ***Behaviour*** | | | |
| | | **Infancy** | **Childhood** | **Adolescence** | **Early adulthood** |
| Bucharest Early Intervention Project (BEIP) | Institutionalized | | worse inhibition of planned action (12 years) | | Lower IQ, lower verbal comprehension, lower processing speed, working memory, perceptual reasoning |
| | | | higher levels of depression and anxiety | | |
| | | | deficits in attention and short-term visual memory, spatial planning and problem solving, spatial working memory, and visual-spatial memory and new learning | | |
| | | Lower IQ | Lower IQ, lower verbal comprehension, lower processing speed | deficit attention and short-term visual memory, spatial planning and problem solving, visual-spatial memory and new learnin | |
| | | | attachment disorders, emotional reactivity, and psychiatric symptomatology, higher psychopathology score | externalizing and internalizing symptoms, worse self-control, higher rates of psychiatric disorders, ADHD | |
| | | | worse self-control | blunted reactivity to social stress | |
| | Adopted | | higher levels of depression and anxiety | higher rates of psychiatric disorders, ADHD | Lower IQ, lower verbal comprehension, lower processing speed, working memory, perceptual reasoning |
| | | Lower IQ | Lower IQ, lower verbal comprehension, lower processing speed | | |
| | | | higher psychopathology score , worse self-control | | |
| | | | deficits in attention and short-term visual memory, spatial planning and problem solving, spatial working memory, and visual-spatial memory and new learning | attention and short-term visual memory, spatial planning and problem solving | |
| | | ***Brain*** | | | |
| | | **Infancy** | **Childhood** | **Adolescence** | **Early adulthood** |
| | Institutionalized | Higher theta power, lower alpha and beta power | less alpha power and a higher proportion of theta power | less alpha power and a higher proportion of theta power | |
| | | | smaller brains overall, reduced white matter and grey matter volumes | thicker cortex in medial (mPFC), inferior frontal gyrus (IFG), temporal pole | |
| | | | | decreased white matter integrity in the superior longitudinal fasciculus | |
| | Adopted | Higher theta power, lower alpha and beta power | smaller brains overall, reduced white matter and grey matter volumes | thicker cortex in medial (mPFC), inferior frontal gyrus (IFG), temporal pole | |
| | | ***Behaviour*** | | | |
| | | **Infancy** | **Childhood** | **Adolescence** | **Early adulthood** |
| English and Romanian Adoptee (ERA) study | Adopted (more than 6 months in institutions) | | ASD, disinhibited social engagement, ADHD | ASD, disinhibited social engagement, ADHD | |
| | | | higher rates of cognitive impairment | higher rates of cognitive impairment | |
| | | | | | emotional symptoms |
| | | | | | worse IQ, prospective memory, proactive inhibition, decision-making, and emotion recognition |
| | | | | | higher prevalence of mood disorders |
| | | ***Brain*** | | | |
| | | **Infancy** | **Childhood/adolescence** | | **Early adulthood** |
| | Adopted (more than 6 months in institutions) | | | | smaller total brain volumes |
| | | | | | lower right inferior frontal cortex surface area and volume |
| | | | | | higher right inferior temporal lobe thickness, surface area, and volume |
| International Adoption Project | adopted from institutions (12 months in institutions) | | impaired cognitive flexibility, inhibitory control, and working memory, and perhaps experienced delays in theory of mind development | smaller total volume and PFC, hippocampus | |
| | | | increased risk of *attention problems* , higher Social problems | | |

***Table I**. Overview of the long-term effects of early institutionalization, as discussed in the studies presented in this section of the chapter. ©Dr. Alice Massera*

## Project description

The research presented in this thesis forms part of a larger project aimed at understanding how early adverse experiences impacts social cognition, behavior, and related brain functions in macaques. This initiative strives to reveal the underlying mechanisms linking early psychosocial deprivation to negative outcomes, such as heightened anxiety and ADHD-like symptoms. The study utilizes a macaque model that specifically involves peer-rearing conditions to investigate the effects of early psychosocial deprivation. Macaques are a valuable model for studying early social adversity (ESA) due to their strong parallels with humans across various domains, including social, cognitive, emotional development, and physiological and genetic similarities (Phillips et al., 2014; Rosati et al., 2016; Sallet, 2022; Wang et al., 2020; Xue et al., 2016). In addition, their brain structure and developmental processes closely resemble those of humans, making it feasible to conduct comparative studies on the effects of early social adversity on the brain (Balint et al., 2021; Kim & Kret, 2022; Schumann et al., 2019). Unlike studies on human populations, research involving macaques offers the advantage of better control over early social environmental variables, enabling more precise investigation of the effects of ESA. Furthermore, macaques have a shorter developmental trajectory compared to humans, making it more feasible to conduct longitudinal studies across different life stages.

Previous studies have demonstrated that ESA significantly impacts macaque development in ways that are comparable to human outcomes (Balint et al., 2021; Kim & Kret, 2022; Schumann et al., 2019). These studies also highlight associations between ESA and alterations in brain structure and function (Kalin et al., 2007; McKeon et al., 2022; Raper et al., 2013; Rayson et al., 2021; Vanderwert et al., 2015). However, much remains unknown regarding the long-term effects of ESA in later stages of life, as the majority of research has concentrated on early developmental periods. This study, therefore, presents a unique opportunity to explore the longitudinal effects of ESA on macaque development, particularly during adulthood, a stage that has been relatively understudied.

### Subjects

The study included a sample of 20 rhesus macaques (9 males and 11 females). During infancy, the macaques were divided into two groups: one group was raised by their mothers (n = 10, labeled as 'mother-reared'), while the other group was reared in a peer-only nursery environment, isolated from their mothers and other adult macaques from birth (n = 10, labeled as 'peer-reared'). The peer-reared macaques did not experience the typical early social interactions, such as mother-infant bonding, that are crucial during early development (Ferrari et al., 2009; Maestripieri et al., 2009). Nursery-rearing procedures followed the NIH Guide for the Care and Use of Laboratory Animals, and the animals were transported from the United States to France during their juvenile stage. The research was approved by our local ethics committee (05.09.18) and adhered to the French Ministry of National Education, Teaching, and Research regulations regarding laboratory animal care and use

### Housing

All 20 subjects are housed in six enclosures at the CNRS Rousset Primatological Station, where they live in semi-free-ranging conditions. Each enclosure includes both indoor and outdoor spaces, equipped with enrichment features. The macaques are provided with a daily ad libitum diet consisting of specialized monkey pellets, supplemented with fruits and vegetables several times a week. The macaques are divided into five distinct groups, each housed in a separate enclosure. Two are large groups: the first consists of seven adult individuals (one male and six females) along with three young females approximately one year old. The second group includes six adults (one male and five females), as well as three young males of about one year old. Each of these two groups inhabits an enclosure with two interconnected areas. Additionally, there are three smaller groups: one comprising three adult males, and two others, each consisting of two adult males.

### Behavioural Observations

To evaluate behavior within their natural social environment, the animals were filmed using a Panasonic HV-V180 camera with a frame rate of 50 frames per second. Weekly video sessions were conducted over a five-month span, with recordings scheduled every morning from Monday to Friday, each lasting 120 minutes (6 segments of 20 minutes each). This method resulted in approximately 20 recordings per group. Two cameras were strategically positioned to optimize enclosure coverage, and the footage from both cameras was later synchronized to facilitate thorough analysis.

### Video coding

For each animal (the 'focal animal'), three consecutive 20-minute video recordings have been coded and analyzed (the remaining observations are being coded currently). In the first recording, only the final 10 minutes were coded, with the initial 10 minutes serving as a habituation period to the cameras. Consequently, two primary behaviors—behavioral restlessness and anxiety—were coded for each animal over a total of 50 minutes. Behavioral restlessness, a key behavior of interest in this study due to its potential link to ADHD-related markers in macaques (e.g., indicators of hyperactivity), was measured as the rate of transition between different behavioral states (e.g., from resting to locomotion, feeding, grooming, self-grooming and playing; see Coyne et al., 2015). For the anxiety measure, the following behaviors were coded using the focal sampling method: a) self-scratching, self-grooming, yawning, and body shaking, all of which reflect anxiety in macaques (see Coleman & Pierre, 2014; Rayson et al., 2021). To date, videos from one day of observation (3 segments of 20 minutes each) have been manually coded for the twenty animals included in this thesis.

### Statistical analysis

Each 20-minute video was treated as a session, resulting in a total of three sessions per individual (50 minutes of coding per individual). The following behavioral indices were computed based on the group observation coding: a) anxiety frequency, which was calculated by summing occurrences of self-scratching, self-grooming, yawns, and body shakes; and b) restlessness frequency, determined by summing occurrences of transitions between different behavioral states—resting, locomotion, grooming, self-grooming, feeding, and playing. Subsequently, the indices were calculated as follows: anxiety frequency (total frequency of anxiety behaviors divided by the total time coded for each individual) and restlessness frequency (total frequency of restlessness behaviors divided by the total time coded for each individual).

For analysis, a linear mixed model was run with group (mother-reared/peer-reared) as fixed-effects, and subject-specific intercepts as a random effect. Frequency of anxiety and ADHD-like behaviour was used as the outcome measures. For the analysis, a linear mixed model was run with group (mother-reared/peer-reared) as fixed effects and subject-specific intercepts as a random effect. Frequency of anxiety and ADHD-like behavior was used as the outcome measures. The analysis was conducted using R version 4.3.1 (R Core Team, 2023) in RStudio version 2023.03.1+446 "Cherry Blossom" (RStudio Team, 2023). The lme4 package (Bates et al., 2015) was utilized for model fitting, while emmeans (Lenth, 2020) provided estimated marginal means. The car package (Fox & Weisberg, 2018) was employed for hypothesis testing, and ggplot2 (Wickham, 2016) facilitated data visualization. Data analysis was conducted using the dplyr package (Wickham et al., 2023).

## Results

A linear mixed-effects model was used to examine the effect of rearing condition (mother-reared vs. peer-reared) on the frequency of anxiety-like behaviors (Fig.2). There was no significant effect of rearing condition on anxiety frequency ($\beta=-0.026$ SE = 0.037, $t(58)=-0.717$, $p=0.476$). Random intercepts for individual subjects accounted for some variability (SD = 0.059), but the residual variability was larger (SD = 0.099).

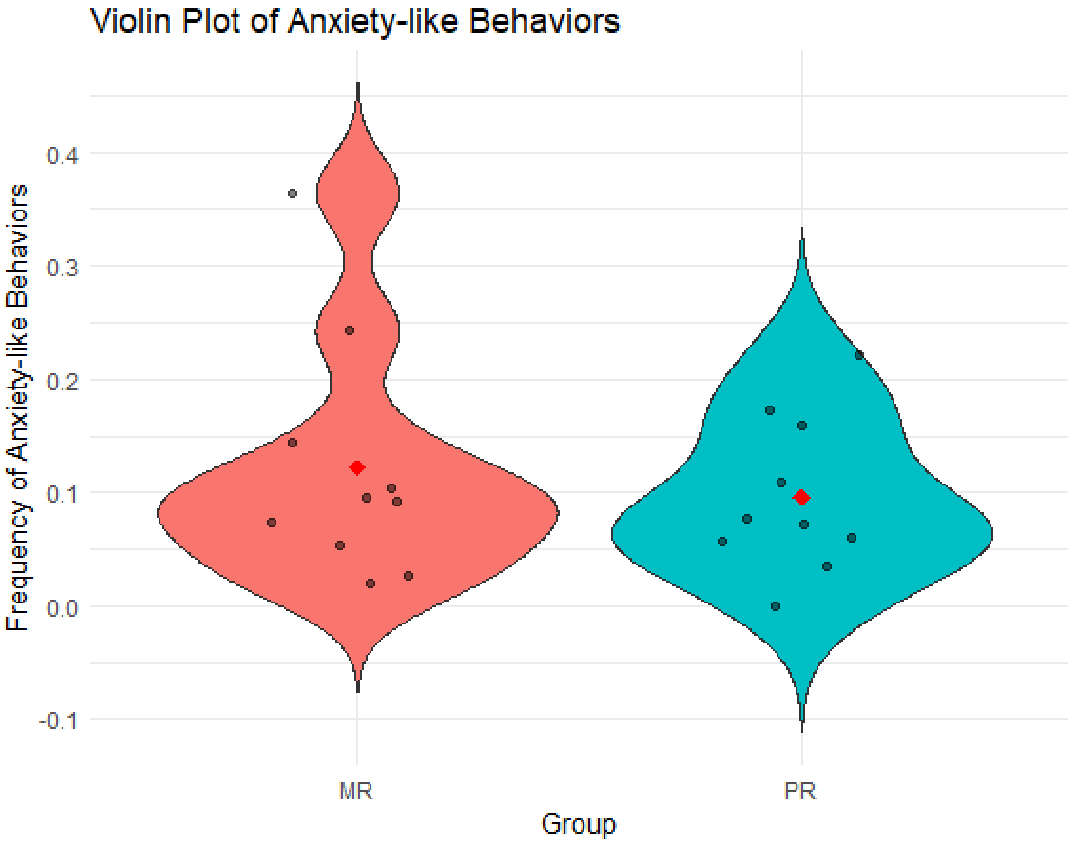

*__Figure 2. Violin Plot of Anxious Behaviors Frequency by Group:__ Violin plot displaying the frequency of anxious behaviors (behavior counts per unit of coded time) across the 'Peer-Reared' (PR) and 'Mother-Reared' (MR) groups. The plot shows the distribution and individual variability of anxious behavior frequencies in each group, along with the mean value. No significant difference in anxious behavior frequency was observed between the two groups.*

A linear mixed-effects model was fitted to assess the effect of rearing condition (mother-reared vs. peer-reared) on restlessness frequency (Fig.3). The effect of rearing condition was not statistically significant (β=−0.030, SE = 0.522, t(58)=−0.058, p=0.954). The random intercepts for individual subjects had a standard deviation of 0.925, while the residual standard deviation was 1.230.

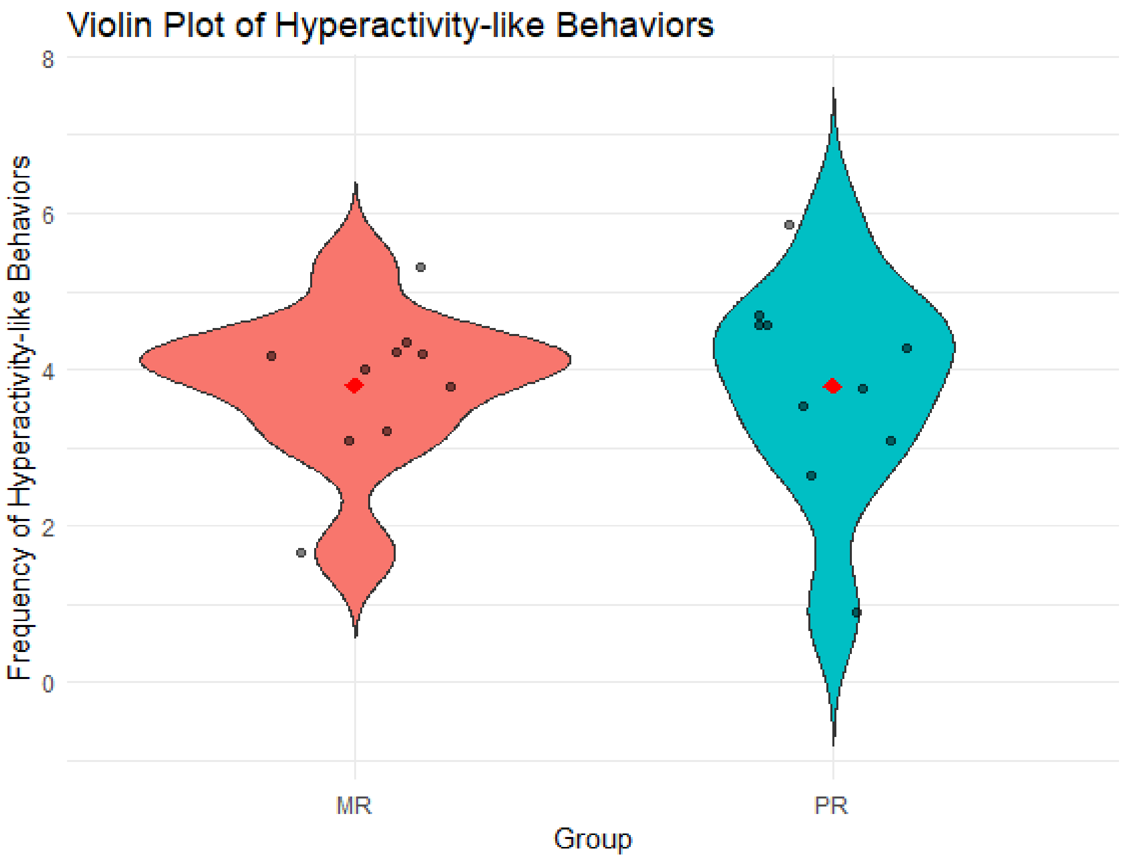

***Figure 3. Violin Plot of Hyperactive Behaviors Frequency by Group:*** *Violin plot illustrating the frequency of hyperactive behaviors (behavior counts per unit of coded time) for the 'Peer-Reared' (PR) and 'Mother-Reared' (MR) groups. The plot depicts the distribution, individual variability, and mean frequency of hyperactive behaviors in each group. No significant difference in hyperactive behavior frequency was found between the two groups.*

## Discussion

Our preliminary findings did not reveal significant differences in behavioral restlessness or anxiety between peer-reared and mother-reared macaques in adulthood. This is in line with the results reported by Rayson et al. (2021) on the same sample, where no difference in anxiety behaviors were found. However, they reported a heightened attention bias toward threats, and this was linked to increased anxiety-like behaviors in pre-adolescent peer-reared macaques. The difference between our findings and those of previous studies is due to the fact that we did not assess any attention bias and our data collections was focused on behavioral observations in their colonies. Thus, potential differences could be due to the subjects' ages, as well as the preliminary nature of our data, which remains inconclusive. Indeed, the analysis is based on a limited number of observations (approximately 50 minutes per individual), while the aim is to code and analyze a significantly larger behavioral dataset.

While a substantial body of research has investigated the effects of early psychological deprivation on human development from childhood into adulthood (Fox et al., 2017; Nelson & Gabard-Durnam, 2020; Rutter, 1998; Zeanah et al., 2003), providing evidence of its impact on anxiety and ADHD-like symptoms (Fox et al., 2017; Rutter, 1998; Zeanah et al., 2003), studies involving macaques have primarily focused on early developmental stages (Balint et al., 2021; Kim, 2022; Schumann et al., 2019). This focus has left a significant gap in our understanding of the long-term consequences of early social deprivation in adult monkeys.

Regarding anxiety, very little research has investigated this phenomenon in fully mature adult macaques (Corcoran et al., 2012). In this study, using the Human Intruder Paradigm, Corcoran et al. (2012) reported that peer-reared adult macaques exhibited inhibited behaviors, including reduced locomotion, decreased exploratory activities, and increased freezing. These results are consistent with earlier findings that showed greater behavioral inhibition in younger peer-reared macaques (Capitanio et al., 2006). However, our results did not replicate these outcomes in adult peer-reared macaques. As noted earlier, our findings are preliminary and should be interpreted with caution. One potential reason for the differing results may be the variations in methodologies used to assess anxiety-like behaviors (e.g., the Human Intruder Paradigm versus behavioral observations in a more naturalistic setting). Additionally, as seen in studies of human aging—where anxiety often declines with age, possibly due to improved emotional regulation (Chaudhary et al., 2023; Charles & Carstensen, 2010)—a similar pattern may occur in macaques.

Concerning the restlessness behavior examined in this study, this is not the first time it has been investigated using the macaque as a model. For example, a previous study explored a polymorphism in the dopamine receptor D4 (DRD4) gene, which is linked to ADHD in humans, and found an association between specific genotypes and levels of 'behavioral restlessness' (Coyne et al., 2015; Tovo-Rodrigues et al., 2013; Qian et al., 2018). However, to our knowledge, this is the first study to directly compare behavioral restlessness between macaques reared by peers and those raised by their mothers.

In human research, children raised in institutional care have been shown to display increased theta activity and decreased alpha and beta power, patterns that are associated with ADHD symptoms (Marshall et al., 2004; McLaughlin et al., 2010), compared to non-institutionalized children, with these differences persisting into adolescence (Debnath et al., 2020; Vanderwert et al., 2010, 2016). In our study, we did not observe outcomes similar to those found in humans. Once again, these results are preliminary and should not yet be over-interpreted, but they may be explained by the notion that ADHD symptoms often diminish in adulthood for some individuals, although they persist in others (Spencer et al., 2007).

In the next stages of this research, we will code and analyze a substantially larger behavioral dataset. This expanded dataset will be systematically compared to the behavioral data collected by Rayson and colleagues (Rayson et al., 2021) from the same colony of macaques during adolescence and early adulthood. This comparative approach will allow us to better investigate behavioral changes across distinct developmental stages.

In addition to group-level comparisons, we plan to perform individual-level analyses, acknowledging that group-level assessments may miss subtle differences that only emerge in specific individuals within groups (see Fig. 4). Lastly, an additional analysis will be conducted, dividing the peer-reared individuals into two groups based on their rearing conditions: the surrogate-reared group, consisting of individuals raised in isolation with a surrogate, and the peer-reared group, comprising those raised with peers but without adult individuals. Given the preliminary nature of our current findings, further investigations are essential to determine whether the effects of early social adversity (ESA) have long-term repercussions in later life stages of macaques, which will be important for guiding future research with humans.

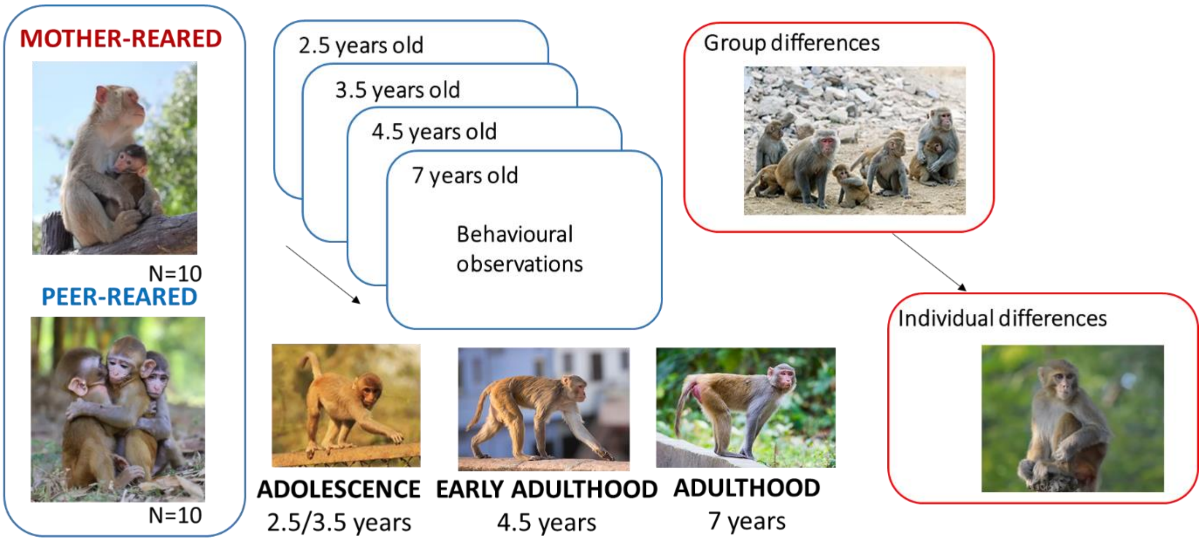

***Figure 4. Illustration of the subsequent steps in the project:*** *(left) a comparison of the behavioral data from the adulthood life stage between the two groups and the behavioral data from earlier developmental years (from pre-adolescence to pre-adulthood); (right) an analysis of differences not only at the group level but also at the individual level.*

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# Posters and communications

- Annicchiarico G., Belluardo M., Vallortigara G., Ferrari P.F. (2022) Do macaques count from left to right? - Poster presented at the Workshop « Comparative Neurobiology of Higher Cognitive Functions » held in Erice from September 9th to 14th, 2022.
- Annicchiarico G., Belluardo M., Vallortigara G., Ferrari P.F. (2023) Number-Space Association in Macaques and the Influence of Emotions – Poster Presented at the Workshop « Ethology Past, Present and Future » held in Erice from May 3th to 6th, 2023.
- Exploring Number-Space Association and the Influence of the Emotional Context in Macaques - Oral presentation at the University of Parma on June 21, 2023.
- Baldi J., Disarbois E., Coude G., Mendez C. A., Maigre L., Annicchiarico G., Cuvilliez M., Duhamel J.-R.,. Ferrari P. F. (2023) Neuronal activity in prefrontal and ventral premotor cortex linked to social affiliative behaviors in free-moving macaques – Poster presented at the Society for Neuroscience Annual Convention (SfN) held in Washington D.C