
**The neural representation of
numerosity zero
in the parieto-frontal magnitude network**

Dissertation

zur Erlangung des Grades eines
Doktors der Naturwissenschaften

der Mathematisch-Naturwissenschaftlichen Fakultät
und
der Medizinischen Fakultät
der Eberhard-Karls-Universität Tübingen

vorgelegt von
Araceli Ramírez-Cárdenas
aus Mexiko Stadt, Mexiko

2016

Tag der mündlichen Prüfung: 25.11.2016

Dekan der Math.-Nat. Fakultät: Prof. Dr. W. Rosenstiel

Dekan der Medizinischen Fakultät: Prof. Dr. I. B. Autenrieth

1. Berichterstatter: Prof. Dr. Andreas Nieder
2. Berichterstatter: Prof. Dr. Uwe Ilg

Prüfungskommission:

Prof. Dr. Andreas Nieder
Prof. Dr. Ziad Hafed
Prof. Dr. Cornelius Schwarz
Prof. Dr. Uwe Ilg

Ich erkläre, dass ich die zur Promotion eingereichte Arbeit mit dem Titel:

*The neural representation of numerosity zero
in the parieto-frontal magnitude network*

selbständig verfasst, nur die angegebenen Quellen und Hilfsmittel benutzt und wörtlich oder inhaltlich übernommene Stellen als solche gekennzeichnet habe. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Mir ist bekannt, dass die falsche Abgabe einer Versicherung an Eides statt mit Freiheitsstrafe bis zu drei Jahren oder mit Geldstrafe bestraft wird.

I hereby declare that I have produced the work entitled ".....", submitted for the award of a doctorate, on my own (without external help), have used only the sources and aids indicated and have marked passages included from other works, whether verbatim or in content, as such. I swear upon oath that these statements are true and that I have not concealed anything. I am aware that making a false declaration under oath is punishable by a term of imprisonment of up to three years or by a fine.

Tübingen, den

Araceli Ramírez-Cárdenas

Para **Catalina**,

que a través de mis ojos te
sorprendas

The neural representation of numerosity zero in the parieto-frontal magnitude network

0. SUMMARY	3
1. BACKGROUND & RESEARCH QUESTION	4
1.1 A VISUAL SENSE OF NUMBER.....	4
1.2 THE PARIETO-FRONTAL MAGNITUDE SYSTEM	5
1.2.1 <i>Neuroimaging evidence</i>	6
1.2.2 <i>Single-cell neurophysiology</i>	6
1.3 THE CASE OF ZERO.....	9
1.3.1 <i>Zero in human history</i>	9
1.3.2 <i>The developmental psychology of zero</i>	9
1.3.3 <i>Zero in adult behavioral studies</i>	12
1.3.4 <i>Animal notions of absence and zero</i>	16
1.3.5 <i>Neurophysiological studies</i>	21
2. IDENTIFYING A NEURAL CORRELATE OF ZERO	22
2.1 MINIMAL REQUISITES OF A ZERO NEURAL REPRESENTATION.....	22
2.2 GENERAL EXPERIMENTAL APPROACH.....	23
3. MATERIALS & METHODS	25
3.1 SUBJECTS & SURGERY	25
3.2 BEHAVIORAL PROTOCOL	25
3.3 STIMULI SPECIFICATIONS.....	25
3.4 NEUROPHYSIOLOGICAL RECORDINGS	26
3.5 ANALYTICAL METHODS.....	26
4. DO MONKEYS TREAT EMPTY SETS AS ENDOWED WITH NULL QUANTITY?	30
4.1 TASK PERFORMANCE.....	30
4.2 REACTION TIMES	33
5. IS ZERO REPRESENTED IN THE PRIMATE VISUAL SENSE OF NUMBER?	34
5.1 SINGLE-NEURON REPRESENTATION OF EMPTY SETS.....	34

5.2	VIP NEURONS REPRESENT EMPTY SETS AS A DIFFERENT STIMULUS FROM COUNTABLE NUMEROSITIES	35
5.3	BEHAVIORAL RELEVANCE OF SELECTIVE-NEURONS' ACTIVITY DURING THE SAMPLE PERIOD.....	39
5.4	POPULATION TUNING FOR EMPTY SETS IN VIP AND PFC.....	40
5.5	DECODING STIMULUS IDENTITY FROM POPULATION ACTIVITY	43
5.6	LEVEL OF ABSTRACTION OF EMPTY SET REPRESENTATIONS	44
5.7	CONCLUSIONS.....	47
6.	ARE EMPTY SETS PROCESSED AS OTHER NUMEROSITY STIMULI IN THE PARIETO-FRONTAL MAGNITUDE SYSTEM?.....	49
6.1	PERFORMANCE & REACTION TIMES.....	49
6.2	THE DYNAMICS OF NUMBER, SHAPE AND PROTOCOL IN VIP AND PFC.....	49
6.3	TWO SURGES OF NUMEROSITY SELECTIVITY IN VIP	50
6.4	LATE NEURONAL RESPONSES FOR EMPTY SETS	55
6.5	EMPTY SETS ELICIT A DISTINCT TEMPORAL RESPONSE PROFILE	55
6.6	A DYNAMIC SHIFT IN THE POPULATION TUNING TOWARDS A CATEGORICAL REPRESENTATION OF EMPTY SETS	56
6.8	DIFFERENTIAL CONTRIBUTIONS OF PFC AND VIP TO BEHAVIOR.....	59
6.9	CHARACTERIZATION OF EMPTY SET PREFERRING NEURONS	60
6.9.1	<i>A continuum tuning profile of empty-set preferring responses.....</i>	<i>60</i>
6.9.2	<i>Location of empty set preferring neurons.....</i>	<i>63</i>
7.	DISCUSSION.....	64
7.1	MONKEYS TREAT EMPTY SETS AS CONVEYING A NULL QUANTITATIVE VALUE	64
7.2	DIFFERENTIAL TUNING FOR EMPTY SETS IN VIP AND PFC.....	65
7.3	BEHAVIORAL RELEVANCE OF PARIETAL AND PREFRONTAL REPRESENTATIONS OF EMPTY SETS ..	66
7.4	LEVEL OF INVARIANCE OF EMPTY-SET REPRESENTATIONS TO STIMULUS FEATURES.....	67
7.5	NUMEROSITY ZERO IN THE LABELED LINE CODE FOR NUMBER	67
7.6	NUMEROSITY ZERO IN A LOGARITHMIC NUMERICAL SCALING.....	68
7.7	FROM 'NOTHING' TO 'ZERO'	68
8.	SUPPLEMENTARY FIGURES.....	70
10.	REFERENCES	71
11.	ACKNOWLEDGEMENTS	77

0. Summary

A general magnitude system is hosted in the fronto-parietal network. Neurons in this system represent the number of visual items in a collection, but it is unknown whether this system encodes null quantity (zero). We recorded from the ventral intraparietal area (VIP) and the prefrontal cortex (PFC) of monkeys performing a matching task including empty sets and countable numerosities as stimuli. Monkeys treated empty sets according to the null quantity they represent. This was revealed by a behavioral distance effect: monkeys wrongly matched empty sets with numerosity one more frequently than with numerosity two. However, reaction times were longer than expected in empty set trials.

We first explored whether empty sets find a place in the visual sense of number. For that purpose, we analyzed neural activity during the sample epoch, when numerosity is visually available to the monkey. We found that VIP neurons encoded empty sets predominantly as a distinct category from countable numerosities. In contrast, PFC neurons represented empty sets more similarly to numerosity one than to larger numerosities, exhibiting a numerical distance effect. Crucially, only prefrontal neurons represented empty sets abstractly and irrespective of stimulus variations. Moreover, compared to VIP, the sample activity of numerosity neurons in PFC better correlated with behavioral tuning functions and predicted the outcome of empty-set trials. In the context of previous results, this data suggests a hierarchy in the processing from VIP to PFC, along which empty sets are detached from visual properties and gradually positioned in a numerical continuum. These findings elucidate how the brain transforms the absence of countable items, 'nothing', into an abstract quantitative category, 'zero'.

Second, we analyzed the temporal dynamics triggered by the different stimuli in VIP and PFC during the course of a trial. We found that, in comparison to other stimuli, empty sets elicit later neuronal responses and a distinct temporal response profile in the parieto-frontal magnitude system. Particularly, empty set trials are characterized by a late top-down effect from PFC to VIP. Approximately 200 ms after sample presentation, we identified the start of a dynamic shift in the population tuning towards a categorical representation of empty sets, which continues during the delay period. Consequently, in working memory, empty sets are over-represented in comparison to other stimuli. Correspondingly, a higher percent of neurons was classified as empty-set neurons in the delay period.

Altogether, our results provide evidence that prefrontal cortex plays a central role in attaching a quantitative value to the absence of countable items. The dominant role of PFC in the processing of empty sets suggests that zero is treated differently with respect to other numerosities. In the visual sense of number, countable numerosities, as salient stimuli, elicit bottom-up signals from the parietal cortex. In contrast, to be treated as endowed with numerosity zero, empty sets seem to require a top-down signal originating in PFC.

1. Background & research question

The question of how quantity is coded and processed in the brain has been the focus of much research during the last decade. A general magnitude system hosted in the primate parietal and prefrontal cortices is involved in the representation of quantity. Specifically, VIP and dlPFC give rise to a ‘visual sense of number’. This capability allows primates to estimate the number of items in a collection at a single glance, in a perceptual-like way. But, is the absence of countable items represented as numerosity zero in the primate visual sense of number? Zero seems to be a special case. Behavioral studies in humans and other animals show that zero is represented and treated differently from other numbers. Then, is null-quantity encoded and processed in the primate parieto-frontal magnitude system as other numbers are?

1.1 A visual sense of number

Humans can quickly estimate the number of items in a visual collection at a single glance, quickly and without counting (Stanislas Dehaene 1997; Burr and Ross 2008). Importantly, this ability does not rely on stimuli properties which covariate with number, as item density or total area. Numerosity can be extracted independently from other stimulus properties (Park et al. 2015).

Moreover, the fact that infants and animals are able to discriminate numerosities suggests that this capacity is phylogenetically ancient and pre-symbolic in character (Brannon and Terrace 1998; Nieder 2005). Indeed, it resembles primary sensation in important aspects. For example, numerosity is susceptible to adaptation, as sensory properties are (Burr and Ross 2008). More fundamentally, our apprehension of number follows Weber’s psychophysical law (Shepard, Kilpatrick, and Cunningham 1975), a signature of sensory perception.

Weber’s law states that the minimal detectable difference between two stimuli is proportional to their intensity. For example, two relatively heavy objects should differ by a greater amount of weight than two lighter objects in order to be discriminated. So, the heavier two objects are, the harder it becomes to distinguish them. The minimum amount by which stimulus magnitude should differ or change in order to be noticeable in sensory experience (the ‘just noticeable difference’, JND) increases with stimuli intensity. In fact, the ratio between these two factors, the Weber fraction (K), is a constant for each sensory property and modality.

$$(1) \quad \frac{\Delta I}{I} = K, \text{ where } \Delta I \text{ is the JND between two stimuli, and } I \text{ is their intensity.}$$

In the case of number, for two numbers to be distinguished, the required difference between them varies with their magnitude. Notice that Weber's law entails the two behavioral signatures of numerical cognition, referred as the distance and magnitude effects. The **distance effect** describes an improvement in the discrimination of two quantities as the numerical difference between them increases. For example, it is easier to distinguish 2 from 5, than 2 from 3. This is classically evidenced by a decrease in response latencies or an increase in accuracy when items to compare are quantitatively closer to each other. The **size** or **magnitude effect** specifies that, for a constant numerical difference, larger numbers are more difficult to discriminate than small numbers. In this case, comparison reaction times increase and accuracy decreases for larger numbers. For example, it is harder to discriminate between sets with 9 and 10 items, than between sets with 2 and 3 items.

Cognitive scientists frequently elude a 'mental number line' to account for experimental results in the field of numerical cognition. The mental number line is an analogue and spatially-oriented representation of the ordered numbers. There is relative agreement that the number line is oriented from left to right. However, its scaling (linear or logarithmic) and range are still topics of controversy. The scaling of the number line is discussed in the context of how to account for the *size effect* in numerical cognition. For that purpose, the literature offers at least two options: the logarithmic and the scalar variance hypotheses (**Figure 1.1**). In the first case, we could assume that our internal representation of number is non-linearly related to objective number. In this line, the dominant view is that the scaling of the number line is compressive, particularly logarithmic. Numerosity would be represented on a logarithmic scale with a constant level of noise along magnitudes. The second alternative to explain the *size effect* involves a linear scaling of number, but an increasing level of noise for larger magnitudes. According to the scalar variance proposal, the size effect emerges as larger magnitudes are more noisily represented.

1.2 The parieto-frontal magnitude system

In the early 19th century, Gall had already claimed the existence of 'a center' of calculation (Kahn and Whitaker 1991). The involvement of different cortical areas in magnitude estimation and comparison was first revealed by the study of deficits after accidental or pathological lesions in humans. Damage to the parieto-occipito-temporal junction was early identified as a cause of acquired dyscalculia or acalculia (Gerstmann 1940; Henschen 1919). Moreover, posterior parietal lesions in the perinatal period have been pointed out as origin of developmental dyscalculia (Levy, Reis, and Grafman 1999; Isaacs et al. 2001). However, more anterior lesions were also identified as cause of impairment in magnitude processing and calculation. Particularly, it was reported that frontal lesions compromise the ability of patients to

estimate magnitudes in different domains and affect their arithmetic abilities (Shallice and Evans 1978).

1.2.1 Neuroimaging evidence

Later, functional imaging (Roland and Friberg 1985; S Dehaene et al. 1996) and human neurophysiological studies (S Dehaene 1996) confirmed and specified the role of parietal and frontal cortices in magnitude cognition. Particularly, these studies reported bilateral parietal and prefrontal activations when subjects were required to do mental arithmetic. Since then, the intraparietal sulcus has been consistently identified as a crucial area in the processing of digits and calculations. However, the IPS is also activated in tasks involving non-symbolic quantities as sets of dots, series of tones or light flashes (Castelli, Glaser, and Butterworth 2006; Piazza et al. 2004; Piazza et al. 2007; Piazza et al. 2006). Such are examples of non-symbolic presentations of number, commonly referred as '**numerosities**'. It has been shown that attending the numerosity of a stimulus elicits strong bilateral activations of the IPS (Castelli, Glaser, and Butterworth 2006; Piazza et al. 2006). But interestingly, this region encodes numerosity even when it is not behaviorally-relevant; for example, when subjects are passively presented with a rapid stream of sets of dots (Piazza et al. 2004). These findings suggest that numerosity perception is hard-wired in the human brain. Further support for this claim comes from studies in children and infants (Temple and Posner 1998). Parietal activations have been described in four-year-old children attending to the numerosity of visual sets (Cantlon et al. 2006). Moreover, event-related potentials in the right parietal cortex of infants signal changes in numerosity when a stream of sets of dots is presented (Izard and Dehaene 2008).

More recently, high-field fMRI revealed a topographical representation of numerosity in the parietal cortex (Harvey et al. 2013). Importantly, this numerosity map is robust to changes in low-level stimulus features. As a correlate of the numerical *size effect*, the cortical area devoted to each numerosity decreases with increasing numerosity and the tuning width increases with preferred numerosity. This topographical encoding of numerosity in parietal cortex resembles the representational organization of sensory areas, and further supports the characterization of a 'sense of number'.

1.2.2 Single-cell neurophysiology

Any proposed neurobiological substrate for numerical cognition should account for the patterns observed in the way subjects deal with numerosities. Single-cell neurophysiology offers a robust correlate of the psychophysics of numerical discrimination; specifically, it accounts for the *distance* and *size effects*. As shown in

Figure 1.1, the posterior parietal cortex and the lateral prefrontal cortex (PFC) host high proportions of number-tuned neurons (Nieder and Miller 2004). Among the explored cortical areas, lateral prefrontal cortex hosts the highest proportion of numerosity selective neurons. In the posterior parietal cortex, number neurons are more abundant in the fundus of the intraparietal sulcus, and particularly, in the ventral intraparietal area (VIP). Lower proportions of number selective neurons have been also found in the antero-inferior temporal cortex (Nieder and Miller 2004).

The firing responses of these neurons are maximal to a 'preferred numerosity' and gradually drop off as the presented stimulus differs in quantity from such number (Nieder, Freedman, and Miller 2002). This pattern of response is the defining feature of numerical tuning. Importantly, the response of these neurons is not driven by low level stimulus features (e.g. total dot area, dot density) and is not affected by other stimuli appearance parameters (e.g. dot configuration or dot color). Two examples of single neurons tuned to numerosity are shown in **Figure 1.2**.

The responses of subpopulations of numerosity selective neurons, with different preferred numerosities, create overlapping numerosity filters (**Figure 1.3B**). Notice that these filters mirror the animal performance for the different sample stimuli (**Figure 1.3A**). Interestingly, in a linear scaling, these neuronal filters are asymmetrical; only after a logarithmic transformation of the numerical scale they become symmetric Gaussian functions (Nieder and Miller 2003) (Compare **Figure 1.3B** left and right).

At the neuronal population level, the behavioral *distance effect* could be attributed to the overlap between neuronal tuning functions engaged in the discrimination of different numerosities. For a pair of adjacent numerosities, the large overlap between their corresponding filter functions makes the discrimination more difficult (a low signal-to-noise ratio). The numerical *magnitude effect* can be explained by the fact that, on average, neurons are less precisely tuned as preferred numerosity increases (Nieder 2005). For example, notice that the filter curves are narrower for small numerosities and wider for large numerosities when plotted on a linear scaling of number.

Further neurophysiological studies confirmed these findings and extended the domain of such **labeled line code** to other types of magnitudes as lengths (Tudusciuc and Nieder 2007) and proportions (Vallentin and Nieder 2008). Concerning the range of stimuli, neurophysiological studies have shown that numerical tuning in VIP and PFC neurons can account for the discrimination of numerosities up to 30 (Nieder and Merten 2007). In contrast, in the small extreme of the natural numbers the neural correlates of zero have remained barely explored. Does zero find a place in the 'neural number line'?

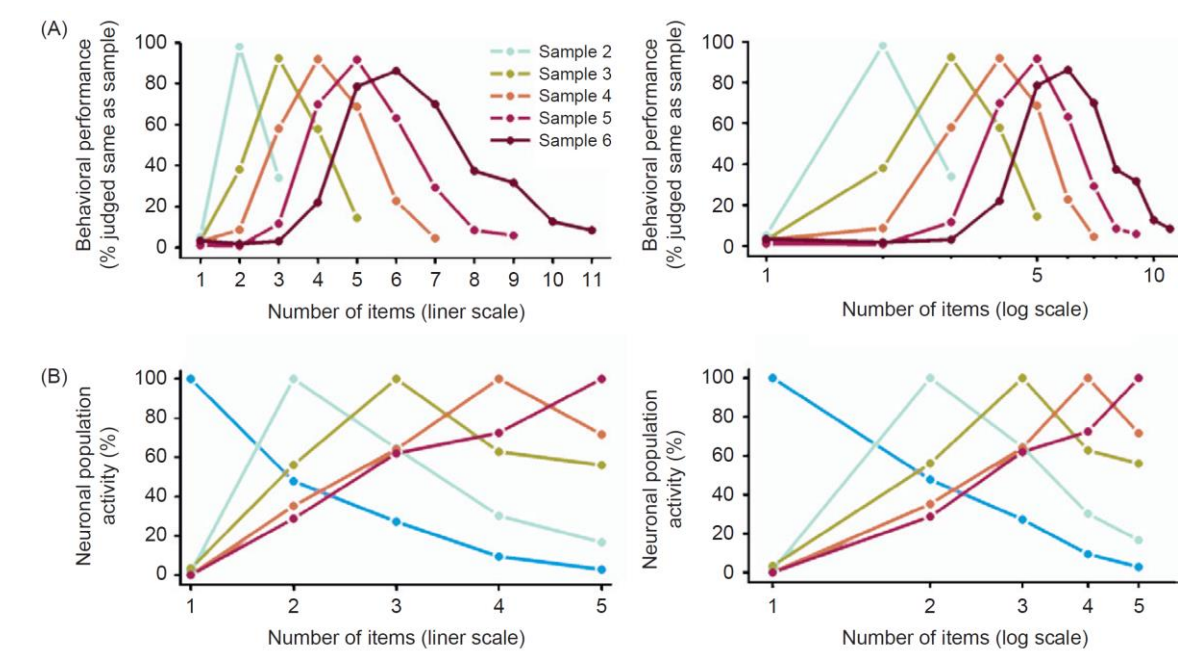
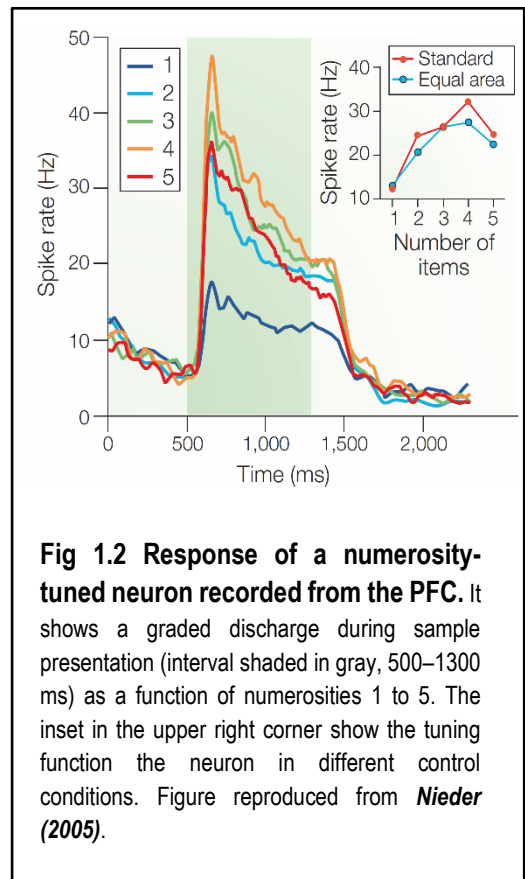
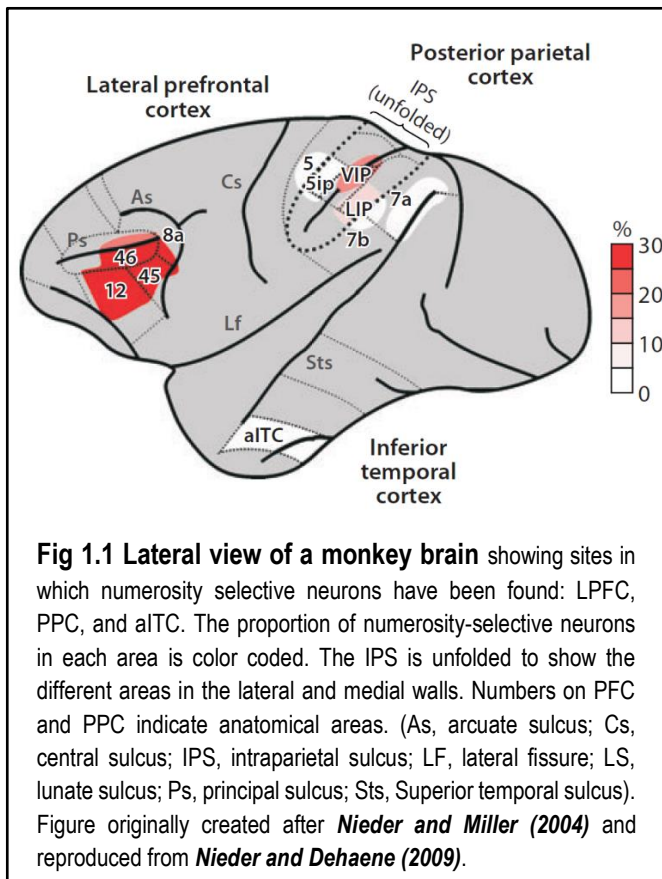


Fig 1.3 Relation between monkey behavior and numerosity-selective neurons. (A) Behavioral numerosity discrimination functions. The curves indicate whether the first test stimulus was judged as containing the same number of items as the sample display. The function peaks (and the color legend) indicate the sample numerosity from which each curve was derived. Behavioral filter functions are skewed on a linear scale (*left*), but symmetric on a logarithmic scale (*right*). (B) Averaged single-cell numerosity-tuning functions from PFC. They are also asymmetric on a linear scale, but symmetric after logarithmic transformation. Originally created from *Nieder and Miller (2003)* and reproduced from *Nieder (2011)*.

1.3 The case of zero

Zero is frequently deemed a special number. In principle, it represents the absence rather than the presence of items to be counted. Therefore, in contrast to positive integers, zero is not a counting number. Indeed, zero could be considered an instance of what is called “negative information”, information conveyed by the absence of a stimulus. Given these singularities, the understanding of zero may rely on different cognitive processes than those supporting the apprehension of other numbers. For instance, it has been suggested that the understanding of zero demands higher representational or symbolic capabilities. To support this hypothesis, zero is depicted as a ‘late comer’: Both in human history and cognitive ontogeny zero is considered a late achievement.

1.3.1 *Zero in human history*

Zero emerged independently at least two times in human history (Seife 2000). In both cases, it appeared as a placeholder symbol in notational systems; namely, as a symbol marking the absence of a proper value in a certain position of the system. Babylonians (300-400 B.C.) first left an empty space in their cuneiform number system and later used a symbol (angled wedges) to mark the empty column. Zero emerged by second time, independently, in the New World during the first centuries A.C: the Mayan culture used a symbol (a shell or closed hand) to denote a placeholder in their vigesimal positional system. However, Indians are credited as the first civilization which deployed a symbol for null-quantity in arithmetic calculations. They are possibly inheritors of the Babylonian use of zero as a place-holder. In any case, the Indian zero would spread through the north of Africa and find its way to Europe around 1200 A.C. with the rest of Arabic numerals.

1.3.2 *The developmental psychology of zero*

In cognitive ontogeny, a parallelism is frequently drawn: children master the cardinal and ordinal properties of small countable numbers before they can deal with zero as a numerical concept (Wellman and Miller 1986). Experiments (Wynn and Chiang 1998) showed that eight-month old infants detected the magical disappearance of a single object leaving an empty set, but failed to detect the magical appearance of an object surreptitiously added to an empty set. Wynn and Chiang argued that infants’ failure in the second condition is related to their incapacity to represent an initial empty set. Particularly, Wynn argues that magnitudes are primarily represented by a numerical accumulator. Given that there is no value for zero in such accumulator, children fail at representing zero.

However, even after children are able to represent the 'absence' of an object, the concept of zero is far from reach. The understanding of zero seems to impose particular cognitive challenges. For instance, one of the 'counting-principles' proposed by Gelman and Gallistel (Gelman and Gallistel 1978), the one-to-one principle, cannot be applied to zero. This principle states that each counting object is assigned its unique symbol (numeral). But the symbol for zero cannot be attached to any object (Wynn 1998).

Wellman and Miller (1986) have proposed a series of stages for the understanding of zero by young children. First, they can verbally identify the symbol for zero without any understanding of what it means. Later, young preschoolers simply treat zero as representing "nothing" or "none", but not yet as numerical concept. Particularly, they do not recognize the magnitude relations between zero and other numbers. For example, in this stage, they are as prone to say that zero is larger than two as vice versa. Moreover, they characteristically insist that one is the smallest number. Only by the end of preschool years, most children understand zero as a numerical concept by recognizing its relations with other numbers. Then, they are able to identify zero as the smallest natural number.

But, could children's initial failure be explained by the cognitive load imposed by the manipulation of numerals? Merritt et al. (2013) have shown that pre-scholar children understand the numerical value of empty sets before they have developed a concept of symbolic zero. Specifically, four-year olds can position empty sets in the context of other small numerosities. In one task (Merritt and Brannon 2013), children were presented two non-symbolic quantities (groups of dots presented in a square background) and required to select them in ascending order in a touch-sensitive screen. Their performance in trials involving empty sets improved as the numerical distance between the two stimuli increased (**Figure 1.4**, continuous line). Merritt et al. compared such distance effect for empty sets with the distance effect for numerosity one (**Figure 1.4**, dotted line with square markers). They reasoned that, if children treat empty sets as numerical values, these distance effects should be similar. Such was the case, and the authors concluded that pre-scholar children possess a non-symbolic notion of zero.

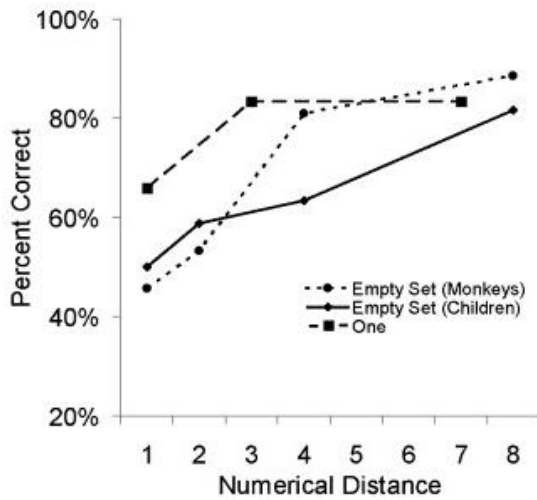


Figure 1.4 Performance for empty sets and one-item sets as a function of numerical distance. For both empty sets and numerosity one, accuracy increases with numerical distance between the pair of presented stimuli. For comparison purposes, the authors included in the figure data from a previous study in Rhesus monkeys (*Merrit et al. 2009*). Figure reproduced from *Merrit & Brannon (2013)*.

However, it is worth noting that children were better at ordering two non-empty sets compared to pairs that included an empty set (**Figure 1.5**). Then, empty sets still represent a challenge for pre-scholar children.

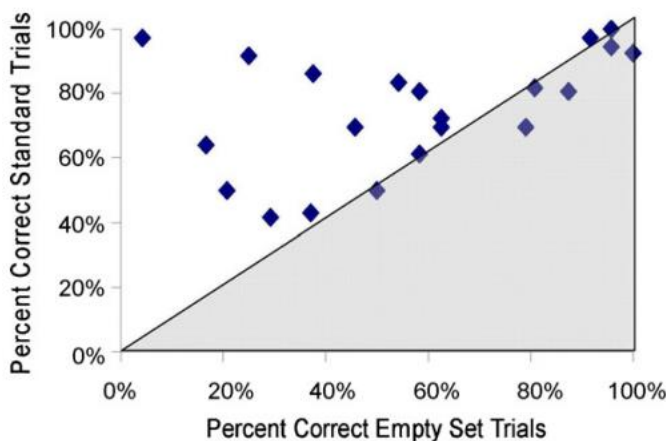


Figure 1.5 Comparison of children's performance on trials involving empty-sets and non-empty set trials. Data from an ordering task with non-symbolic stimuli (sets of dots). Most data points lie in the non-shaded area, indicating better accuracy on non-empty set (standard trials) than on empty set trials. Figure reproduced from *Merrit & Brannon (2013)*.

On other three tasks, the authors tested the children's comprehension of the symbolic zero¹. Interestingly, they found that performance on symbolic-zero tasks was grossly predictive of performance on the ordering of empty sets. These results

¹ Symbolic number tasks included: (1) "Give a number" task: children were presented with 12 plastic dolphins in a pile and asked "Can you give me one dolphin?" A titration procedure was used, so the number of requested dolphins increased by one after each correct response and decreased by one after an error; (2) "How many" task: children were presented with 1-6 plastic dolphins in a line and were asked "How many dolphins are there? Can you count them out loud?"; (3) Smallest number query: Children were asked "What is the smallest number in the world?"; (4) Symbolic ordering task: children were presented with a pair of Arabic numerals written on cards. Then, they were asked "which one is the smaller number?" If a positive integer was offered as answer, the child was asked "Is there a number smaller than that?" The procedure was repeated until the child failed to provide a smaller number.

suggest that children grasp the ordinal relationships between empty sets and other non-symbolic numerosities before they understand how the symbolic zero relates to other numerals. The authors conclude that children can appreciate empty sets as magnitudes on a mental number line, and for that purpose, they do not require comprehending the symbolic zero's numerical meaning.

Finally, other studies have focused on how children use notations to represent quantities; namely, on the development of some symbolic representation of numerosity. For example, Bialystok and Codd (2000) examined this development of quantity representations in children aged 3 to 7 years old using a game-like scenario. Particularly, they analyzed the notations children used to represent different quantities (including zero) and their understanding of those representations. They found that at the age of 3, subjects already represented zero as absence of objects in a consistent way (more than half the trials). However, three-year-olds still find more difficult to deal with zero than with positive whole numbers. At the age of four, children solved problems involving zero as well as they do with other small natural numbers (Bialystok and Codd 2000).

1.3.3 Zero in adult behavioral studies

Even educated adults seem to struggle with zero. Wheeler & Feghali (1983) studied the understanding of zero in 52 pre-service elementary school teachers. They found that these subjects not only exhibited confusion as to whether zero is a number, but also had problems in some calculations involving zero. For example, 75% did not responded correctly to the question 'What is 0 divided by 0?' (Wheeler and Feghali 1983).

However, anomalies in the treatment of zero have been described in more basic tasks, for example, when reading numerals. The reading times of Arabic numerals are affected by their numerical value. Specifically, Brysbaert (1995) found that the time to process integers 1 to 99 increases as a function of the logarithm of the numerical value (Brysbaert 1995)². To arrive to this conclusion, Brysbaert et al. excluded the numeral zero from his main analysis. Indeed, confirming a previous study, reading the numeral zero took more time than expected. For example, the processing time for number zero was longer than the processing time for 1 and even other digits. Based on these results, the authors concluded that the processing of zero could be "based on other principles than those used for integers between 1 and 99".

² In this study, reading times were also predicted by the frequency with which the number was presented and, in some cases, the length of the number name.

Moreover, Brysbaert suggested that the number line does not start with zero, but with one.

Withstanding such evidence, Merrit et al. presented adult subjects with pairs of sets containing 0 to 9 dots. Subjects were requested to select first the smaller stimulus and subsequently the larger stimulus in the pair. As expected, reaction times in correct trials decreased with numerical distance between the stimuli. This *distance effect* was similar in pairs including empty sets compared to sets including numerosity one (**Figure 1.6**).

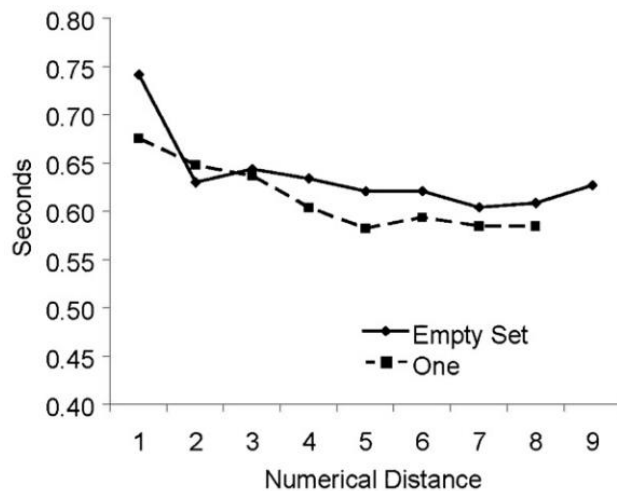
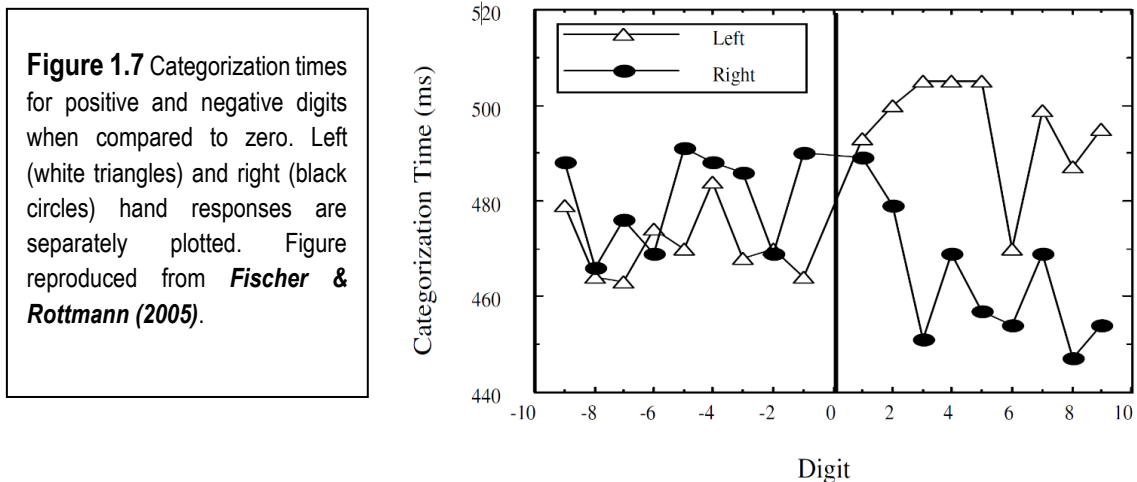


Figure 1.6 Reaction times as a function of numerical distance in adults. Data from an ordering task with non-symbolic stimuli (sets of dots). No significant difference was found between the slope corresponding to empty sets and one. Figure reproduced from *Merrit & Brannon (2013)*.

Most of the evidence that the mental number line is oriented from left to right comes from the association between number magnitude and space. In 1993, Dehaene and colleagues first showed an association between numerical value and personal space. Subjects were asked to press one of two keys in response to an even number and other key to an odd number. They responded faster to smaller numbers with the left hand than with the right hand. The opposite happened for larger numbers (Stanislas Dehaene, Bossini, and Giraux 1993). The systematic interaction between response side and number magnitude was named as 'spatial-numerical association of response codes' (SNARC) and has been robustly replicated in different tasks. The SNARC effect is said to originate when, during a task, the representation of a particular number is automatically activated in the spatially oriented number line.

In their initial study (Stanislas Dehaene, Bossini, and Giraux 1993), Dehaene et al. found shorter latencies in response to number zero with the left hand. This finding suggested that zero is represented in the mental number line as a small number. However, in this and other studies participants frequently doubted about the parity status of zero (Brysbaert 1995; Fias, Lauwereyns, and Lammertyn 2001).

In another study, Fischer and Rottmann (2005) asked participants to decide whether a presented integer (from -9 to 9) was smaller or larger than 0 by pressing a right or left button. They reported a SNARC effect in this task. Moreover, they found that a distance effect was evident when positive numbers were compared to 0, but not for negative numbers (**Figure 1.7**). According to the authors, this finding suggests that the mental number line starts with 0 and does not extend to the left of it (Fischer and Rottmann 2005).

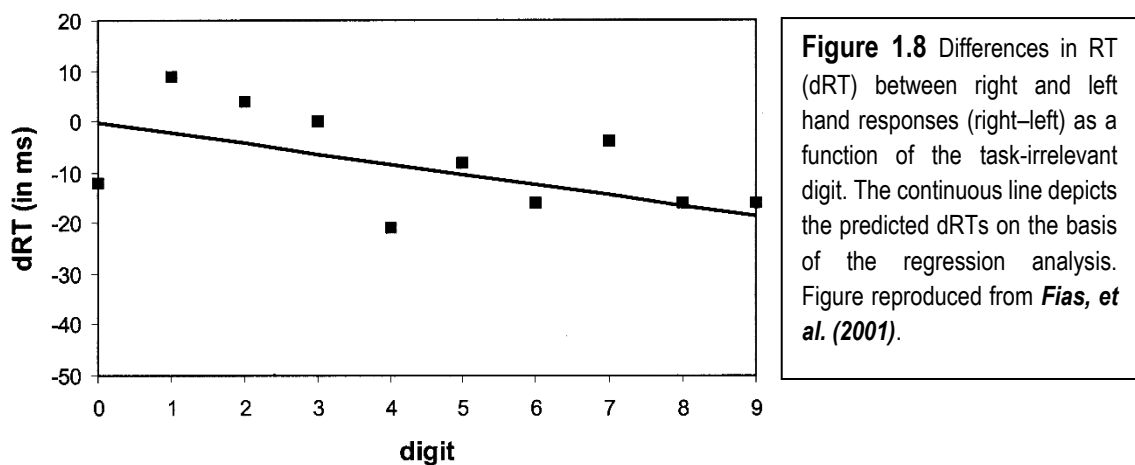


More recently, Pinhas and Tzelgov (2012) studied the automatic processing of numbers using a physical comparison task: Pairs of numerals which differ in physical sizes and magnitude values are presented to subjects who are requested to compare their sizes and ignore their value. In such type of task, faster responses are observed in congruent conditions (when the number which is physically larger is also numerically larger) than in incongruent conditions (when the physically larger number is numerically smaller). This '*size congruity effect*' is measured as the difference in reaction times between incongruent and congruent conditions. The effect generally increases with the numerical distance between the numbers which are physically compared. But crucially, for the smallest number in the range presented the size congruity effect is increased, while its dependence on numerical distance is attenuated. The authors used this '*end effect*' in the automatic processing of numbers to investigate whether zero is represented in the number line. They concluded that, when included in the stimulus range, "0, or 1 in the absence of 0, is perceived as the smallest entity on the mental number line" (Pinhas and Tzelgov 2012).

However, other studies deploying the SNARC paradigm have challenged the inclusion of zero in the mental number line. In the study conducted by Fias et al. (2001), Arabic numerals were displayed in a screen, but were not relevant to solve the task. Instead, subjects had to attend a superimposed cue and report, for instance, if this cue (a triangle) was pointing upwards or downwards, by pressing either the left

or right response button³. The authors found a robust SNARC effect even when numerical value was not task-relevant. They computed differences in reaction times (dRTs) by subtracting the median RT for the left hand from the median RT from the right hand. An association between response side and number magnitude was evidenced by a negative correlation between number value and dRT. Small numbers elicited faster left responses, and therefore positive dRTs. However, in most of the experiments numeral zero elicited more negative dRTs than expected by its null-value (**Figure 1.8**). Based on these anomalies, the authors support the conclusion that “the semantic coding of the number zero might be different than the semantic coding of other numbers” (Fias, Lauwereyns, and Lammertyn 2001).

Exp. 1: Orientation: Triangle pointing up - down



Moreover, a more recent study found that zero is represented separately from other single-digit integers (Nuerk, Iversen, and Willmes 2004). In this study, Nuerk et al. primarily explored the presence of a MARC effect (Linguistic Markedness of Response Codes) for parity: that even numbers are responded faster with the right hand and odd numbers with the left hand. For that purpose, most of their analysis excluded zero. However, they also performed a non-metric multidimensional similarity scaling analysis (MDS) on correlations in the reaction time data across all numbers, including zero, for both response sides. Points depicted closely in the represented space configuration (Smallest Space Analysis, SSA) represent highly correlated stimuli. The authors explain that, if zero is not part of the number line, it should be located far away from other numbers. But, “if it is an ordinary even number, it should be located close to other small numbers and to other even numbers”. Given the results

³ The authors carried out 4 versions of the experiment. In one, referred in the main text, the subjects had to report if the triangle was pointing up or down. In other two versions, they had to report its color (In Experiment 2, green or red; In Experiment 3, light or dark cyan). Finally, in a fourth version, the cue was a line segment and participants reported its orientation (horizontal or vertical).

shown in the **Figure 1.9** below, the authors concluded that such is not the case: zero in an unconventional number, represented far from other small numerosities.

SSA for Positive Arabic Numbers 0 - 8

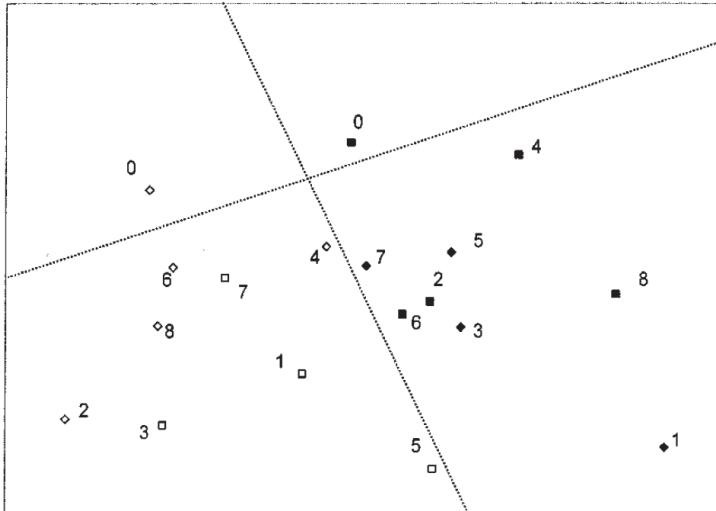


Figure 1.9 SSA for positive Arabic numerals 0 to 8, responded to with the right hand (squares) and the left hand (diamonds). Based on this analysis Nuerk et al. (2004) assert that zero strongly differs from all other numbers regardless of whether it is responded to with the left or the right hand. Figure reproduced from **Nuerk, et al. (2004)**.

1.3.4 Animal notions of absence and zero

Altogether, historical, developmental and behavioral considerations may suggest that zero is not processed in the brain as other numbers are. Moreover, it has been suggested that the concept of zero demands a higher level of abstraction. Particularly, higher symbolic or representational capabilities could be required to apprehend zero. Animals are commonly seen as lacking those capabilities. This belief could explain the scarcity of studies exploring the animal understanding of null-quantity. But, do animals lack even a primitive non-symbolic concept of zero? Given the remarkable commonalities with humans in dealing with non-symbolic quantity, some kind of the precursor for the understanding of zero would be expected in animals.

1.3.4.1 Animal cognition of absences

The understanding of 'absence' seems to precede the apprehension of zero, at least in children. Do animals possess a concept of absence? Pepperberg (1988) trained an African Gray parrot, Alex, in the notions of 'same' and 'different'. Objects with three attributes (color, shape and material) were shown in pairs to Alex. Then, he was questioned "What's same?" or "What's different?" He was expected to mention the property (category label) with respect to which the objects coincided or differed. For example, when shown a red plastic cube and a red wooden sphere, and asked "What's same?", he would reply "color". Later, the paradigm was extended by also showing pairs of identical or totally dissimilar objects, so he could reply "none" to the

questions “What’s different?” or “What’s same?”. After training in this type of questions, Alex was able to transfer his use of “none” to new stimuli: his accuracy was high for pairs of novel objects, and also when unfamiliar colors, shapes and materials were tested. Pepperberg et al. concluded that Alex possessed an abstract notion of ‘absence’, which relied on the violation of an expectation of presence (Pepperberg 1988).

1.3.4.2 A zero-like concept in animals?

A later study (Pepperberg and Gordon 2005) reported that Alex transferred his use of “none” to a numerical task. In this occasion, a set of differently colored blocks was presented. For each color, the number of blocks in the set ranged from 1 to 6. Alex was asked about the color which corresponded to a particular number. For example, if shown four blue, three red, and two green blocks and questioned “what color four?” Alex would reply “blue”. Surprisingly, when by mistake Alex was questioned for the color of a non-present numerosity, he spontaneously answered “none”. According to the authors, this spontaneous transference in the use of “none” shows Alex is able to label a null set. Controversially, from these results, the authors asserted that Alex possessed a ‘zero-like concept’.

In a follow-up study (Pepperberg 2006), concerning addition, Alex was sequentially shown the content of two cups, and later asked the total number of revealed objects. He showed some competence in summing small quantities. However, he failed to say “none” when asked how many items were located under two sequentially presented empty cups. In total, eight of these trials were presented to Alex. In five of those trials Alex refused to answer; in the other three, he said “one”.

As noted by Pepperberg, Alex response of ‘one’ is interesting given that he was never trained on ordinality and learned the numbers in random order. However, he seemed “to grasp that ‘none’ and ‘one’ represented the lower end of a number spectrum”. Nonetheless, Pepperberg recognized that these results expose the limits in the correlation between the use of ‘none’ and the concept of zero. It seems that Alex was using “none” to describe the absence of a particular attribute associated with a collection of objects, but was unable to use it to describe the absence of items themselves.

1.3.4.3 A primate precursor of zero

There is accumulating evidence that non-human primates can recognize and deal with empty sets as numerically significant. Biro et al. (2001) had trained an adult chimpanzee, Ai, to correlate sets of dots to numerals 1 to 9. When the chimpanzee was already competent in matching visual numerosities and symbols, number 0 was introduced to her repertoire. Then, the animal was required to correlate empty sets

with the numeral 0, and viceversa. This task explored the cardinal dimension of zero in the context of other numbers. Ai was competent in the use of zero in this task. However, the main purpose of the study was to determine whether the subject could transfer between the cardinal and ordinal meanings of zero. Therefore, in a subsequent task, they required Ai to sequentially select presented numerals in an ascending order. Without specific training in the ordinal task, the animal ordered the numeral zero in the context of the other numbers. Then, Ai was able to acquire the meaning of zero in the ordinal domain, through training in the cardinal domain (Biro and Matsuzawa 2001).

The authors report that, during training and testing, zero seemed to be progressively shifted towards the lower end of the continuous numerical scale. This was evidenced by a decrease, with training time, in the average value of the labels that she erroneously matched to the zero stimulus. A similar pattern was observed in the ordering task: errors involved progressively smaller numbers. In the end, confusions with 1 remained the most frequently encountered errors. For example, at the end of the testing period, Ai still made the mistake of selecting 1 before 0 in the ordering task. According to the authors, this error pattern suggests that Ai understood the positioning of zero in relation to other number symbols. Such pattern, they argue, is not consistent with an “absence of items versus presence of items” scheme.

In another study (Merritt, Rugani, and Brannon 2009), two Rhesus monkeys spontaneously treated empty sets according to the (null) quantity they represent in tasks involving the discrimination and comparison of visually displayed sets of dots. In the training stage of the study monkeys were shown a sample array of dots (comprising numerosities 1 to 12) and were subsequently required to select between two test arrays the one that matched in numerosity (target stimulus) with such sample. Subsequently, in the testing phase of the match-to-sample experiment, empty set stimuli were inserted in non-differentially reinforced trials; namely, trials containing empty sets were rewarded with juice either after selection of the target (match) or the distractor (non-match) stimulus. This was done to prevent learning and to avoid the extinction of potential behavioral effects. Additionally, experimenters performed controls for stimulus color and free-background area. Both monkeys were successful at matching empty sets without previous training and independently of the stimulus appearance (color or background area). Crucially, for both empty sets and numerosity one, accuracy increased as a function of numerical distance between the tested numerosities (target and distractor) (**Figure 1.10**).

Concerning reaction times, the authors could only conclude that empty sets were treated similarly to numerosity one: One monkey showed a significant decrease in RT with numerical distance for both empty sets and one, while the other monkey failed to exhibit a distance effect for both stimuli.

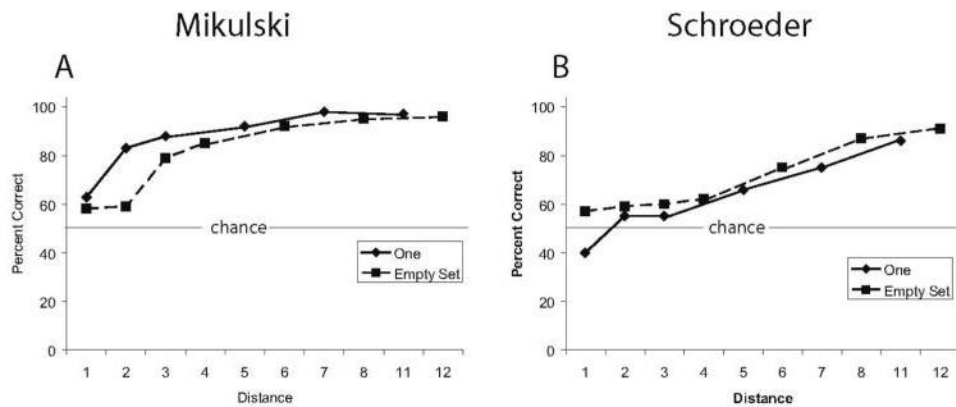


Fig 1.10 Match-to-sample task: Monkeys' accuracy on trials including an empty set or numerosity one. In both monkeys, performance accuracy improves with numerical distance between the target and the distractor numerosity. Figure reproduced from *Merritt, et al. (2009)*.

In a second experiment, monkeys were trained to select the numerically smaller of two dot arrays displayed simultaneously. Empty sets were introduced as stimuli after monkeys were competent in the comparison of numerosities 1 to 12. In trials including empty sets, juice reinforcement was given regardless of the order in which stimuli were selected. Controls for image-like features were also performed for this task. Results show that both monkeys successfully ordered empty sets with respect to other numerosities. Again, their accuracy exhibited similar *distance effects* for empty sets and numerosity one (**Figure 1.11**). This pattern in monkeys' performance further suggests that empty sets were treated as values on a numerical continuum. However, also in these case reaction times were less consistent.

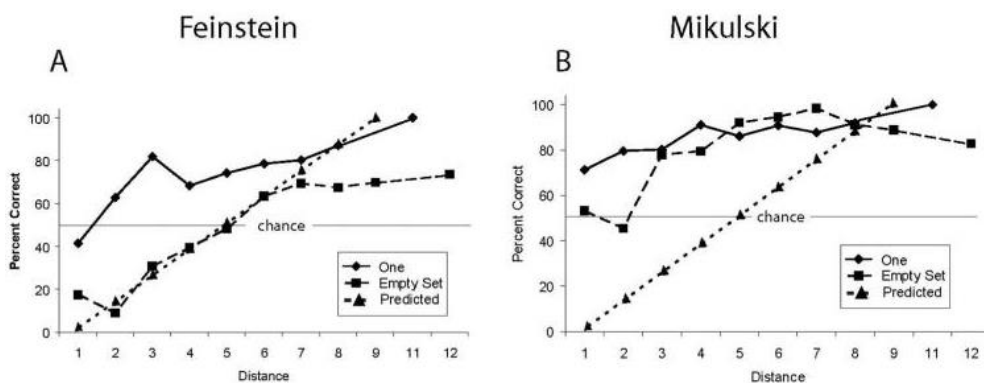


Fig 1.11 Comparison task: Monkeys' accuracy on trials including an empty set or numerosity one. In both monkeys, accuracy improves with numerical distance between the two simultaneously displayed stimuli. Figure reproduced from *Merritt, et al. (2009)*.

More recently, while studying the comparison of auditory and visual quantities by chimpanzees, Beran, (2012) found behavioral indications that empty sets were recognized and appropriately accommodated in the context of other quantities (Beran

2012). In a first experiment the animals were required to choose between two sets of food items (quantity range from 1 to 5) that were auditorily presented. The experimenter dropped candies at a pseudorandom rate through an opaque tube, first into one container and then into another. In the test phase the chimpanzees were expected to choose the container with the larger amount of food items. As predicted, their responses showed the ratio-effect commonly present in numerosity judgments.

In order to exclude possible confounding factors, the authors conducted a second experiment in which one opaque container was replaced by a transparent plastic bowl and a complete set of items was placed into it before starting each trial. Additionally, the experiment included empty sets in the sequential-auditory presentation. Interestingly, when making an error monkeys had more likely selected the auditory set over the visual set. Of this errors 85% involved auditory sets of zero, one or two items. When zero items were presented in the auditory set the chimpanzee incorrectly selected that set in 79.2% of the trials in which one visible item was presented, 25% over 2 visual items and 8.33% over 4. This pattern in the error rate of comparisons involving zero items evidences, again, a *distance effect* for empty sets. Additionally, as noted by the authors, this behavior pattern cannot be explained by the systematic selection or avoidance of any specific visual or auditory set, but only by the number of items in both sets.

It is worth noting that previous primate studies which used food as stimuli included 'zero' as 'absence of food' in tasks involving arithmetic manipulations (Rumbaugh, Savage-Rumbaugh, and Hegel 1987). Probably the first example corresponds to a summation study in chimpanzees conducted by was Rumbaugh et al. (1987). Later, in 1989, Boysen & Berntson reported that the chimpanzee Sheba found no difficulty in learning to match an empty food tray to the numeral 0. Furthermore, the animal was capable of performing addition of numerals ranging from zero to four (Boysen and Berntson 1989).

In another study (Olthof, Iden, and Roberts 1997), squirrel monkeys learned to match Arabic numerals including zero (0,1,3,5,7,9) with corresponding quantities of food. Monkeys were asked to choose one of a pair of these numerals and received the corresponding number of peanuts. At this stage, the correct behavior involved not choosing zero. But later, in an addition task, monkeys correctly chose the larger of two sums (represented by two cards with one to three numerals printed on each of them) even when zero was among the displayed stimuli.

Taken together, studies in non-human primates suggest that these animals can treat empty sets as representing null quantity. Crucially, it has been shown that primates can position empty sets in the numerical continuum and therefore, could possess a non-symbolic primitive representation of zero.

1.3.5 Neurophysiological studies

Recently, Merten and Nieder (2012) studied how the judgment of the presence or absence of a stimulus is represented in the prefrontal cortex (PFC). They recorded prefrontal single-neuron activity in monkeys performing a visual detection task, in which decision was dissociated from motor preparation. As expected, they found neurons that actively encoded the subjective decision about the presence of a stimulus. But surprisingly, after the stimulus was presented and before the monkeys could plan their response, they found a second population of neurons that actively responded to the judged absence of stimuli. Such neurons, named 'no-neurons', significantly changed (in most of the cases, increased) their discharge rates in the delay period, whenever the monkey decided to report the absence of stimulus, both in correct rejections and miss trials (Merten and Nieder 2012).

In addition, preliminary results from one monkey suggested that VIP neurons signal the lack of countable items (Okuyama, Kuki, and Mushiake 2015). However, the invariance of these neuronal responses to low level (i.e. luminance) and appearance stimulus features was not directly tested. Moreover, note that the concept of 'zero' is not exhausted by the notion of 'absence' or 'nothing'. The latter is a binary notion, lacking the quantitative dimension that defines numerosities. As previously suggested, zero is defined by its (ordinal or cardinal) relations to other numbers. Then, which are the neural processes that enable primates to translate absence in to quantity zero?

2. Identifying a neural correlate of zero

Behavioral evidence advances the hypothesis that zero could find a place in the primate visual sense of number. If Rhesus monkeys are able to deal with empty sets as endowed with null quantity, how is such a capacity implemented in the brain? Is zero represented and processed in the parieto-frontal magnitude system as countable numerosities are? To approach these questions, we simultaneously recorded neuronal activity in VIP and PFC while two monkeys performed a numerosity discrimination task, in which empty sets were included in the stimulus pool.

2.1 Minimal requisites of a zero neural representation

The present study was specifically designed to explore the parieto-frontal encoding of empty sets as endowed with a numerosity of zero. Neuronal activity, either at the single-cell or population level, must satisfy three conditions to be considered a correlate of numerosity:

First and fundamentally, numerosity representations **reflect the cardinal relationships between numbers** (Nieder, Freedman, and Miller 2002). Such type of *quantitative tuning*, can be evidenced by the presence of either a '*numerical distance effect*' or a '*numerical size effect*' (Nieder, Freedman, and Miller 2002; Nieder and Miller 2003). As previously mentioned, the *distance effect* describes an improvement in the discrimination of two quantities as the numerical difference between them increases. The *size effect* is reflected by a decrease in the discrimination of larger numerosities compared to smaller numerosities, even when separated by equal numerical distances.

Second, numerosity representations are **invariant to low level stimulus properties** which can correlate with number (i.e. total item area, item density). In the case of non-controlled stimuli, these low level properties could emulate a *quantitative tuning* in neuronal responses. Ruling out this possibility is crucial in numerosity studies.

Third, numerosity representations exhibit some degree of **invariance to stimuli specificities**. In the case of visual stimuli, these would be image-like features, which do not correlate with number (i.e. color or shape of the countable items). Note that a group of three blue stars and a group of three red apples are both instances of three-item-sets. A neuronal response to numerosity three is expected to be similar in both cases. This requisite guarantees a high-level of abstraction in numerical representations.

2.2 General experimental approach

Two monkeys were trained in a delayed-match-to-sample numerosity discrimination task involving as stimuli sets of black dots presented against a gray background (**Figure 2.1**). Stimuli contained from 0 (empty sets) to 4 dots.

To ensure that the monkeys solved the task by judging discrete quantity, low-level visual features were controlled in two stimulus protocols. In the standard protocol, black solid dots appeared at randomized locations and their diameter was pseudo-randomly varied. In the control protocol, overall dot area, dot density and total stimulus luminance were kept constant across countable numerosities (1-4). Background luminance was varied across and between protocols to control for luminance differences that may occur for the empty set, and to detect their effect on neuronal responses (**Figure 2.2**). To test how invariant the neuronal representation of empty sets is to image-like features, both stimulus protocols (standard and control) were shown either with a circular or a square background. If a neuron or a population of neurons are said to represent empty sets quantitatively, they should do it independently of background shape, as both an empty circle and an empty square are instances of numerosity zero.

We recorded simultaneously in the ventral intraparietal cortex (VIP) and the dorsolateral prefrontal cortex (PFC), while two monkeys performed the delay-match-to-sample task (**Figure 2.3**).

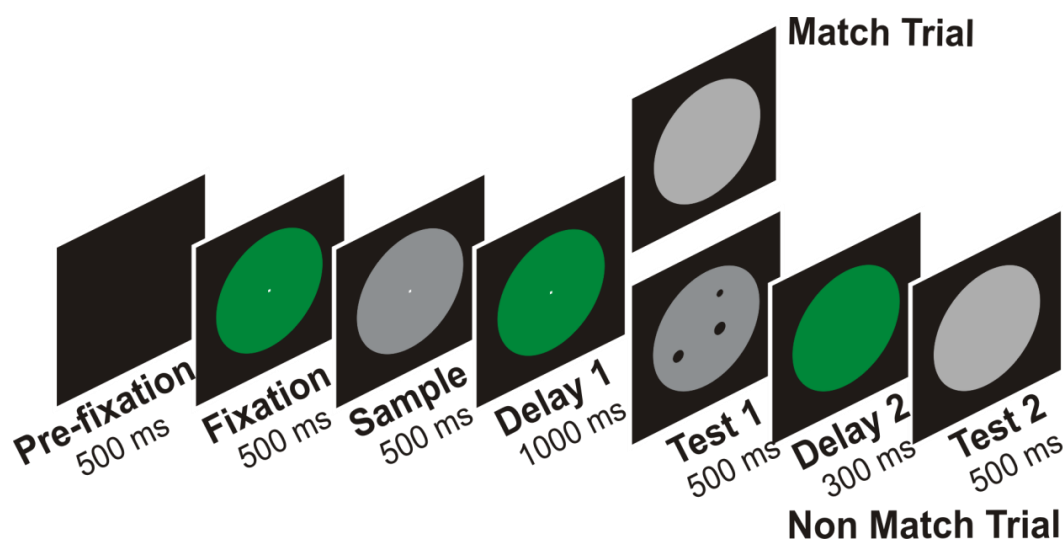


Figure 2.1 Task. Fixating monkeys were presented with a sample numerosity ranging from 0 to 4 for 500 ms. Monkeys had to keep the sample numerosity in memory for a 1-s delay period and match it to a subsequent test stimulus (either the first or the second test stimulus was correct) by releasing a lever.

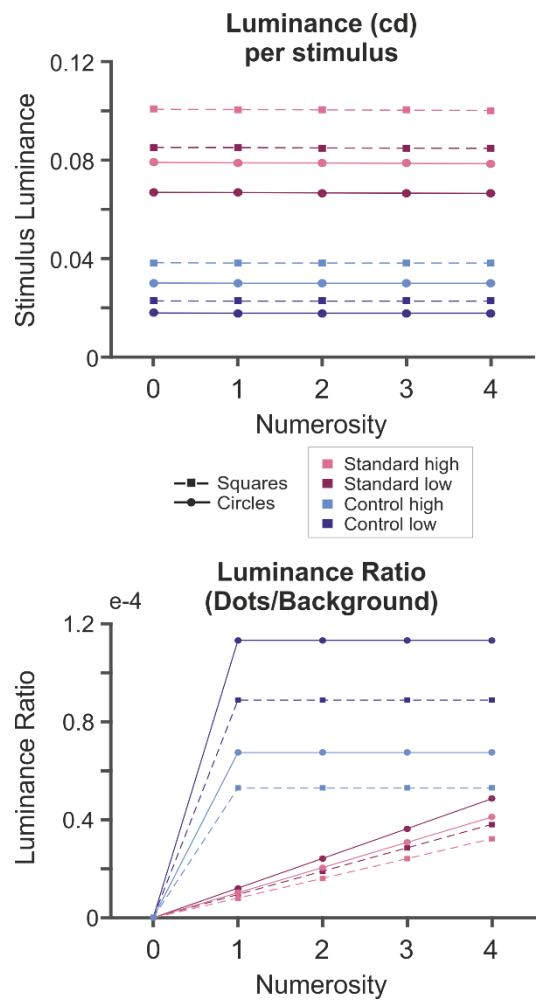
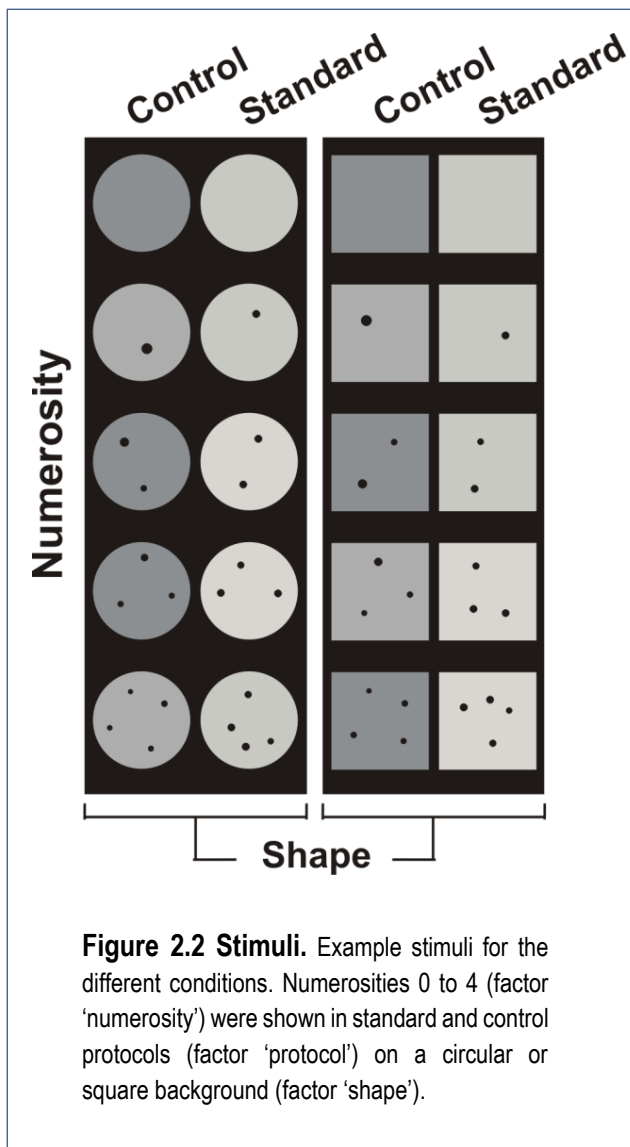
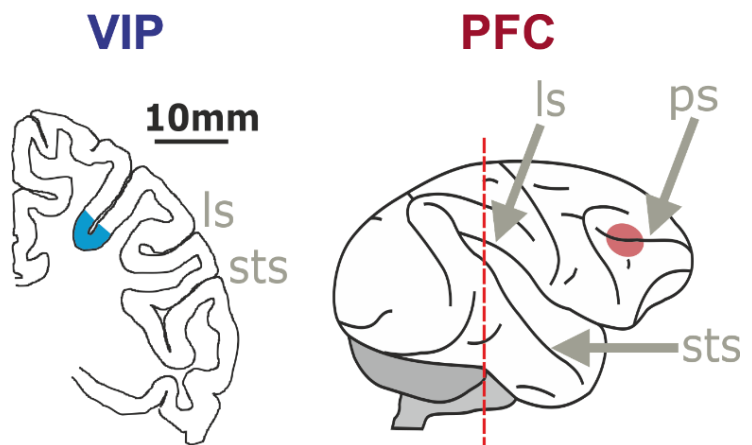


Figure 2.3 Recording sites. Lateral view (right) of the right hemisphere of a monkey brain indicating the topographical relationships of cortical landmarks. Coronal section (left) at the level of the dotted line in the lateral view reconstructed from a structural MRI scan. Red region on the frontal lobe and blue region in the fundus of the IPS mark the recording areas in PFC and VIP, respectively. ips, intraparietal sulcus; ls, lateral sulcus; sts, superior temporal sulcus.



3. Materials & Methods

3.1 Subjects & Surgery

Two male adult rhesus monkeys (*Macacca mulatta*) weighting 6.7 and 8.5 kg were implanted with 2 recording chambers each, centered over the principal sulcus in the dorsolateral prefrontal cortex and the VIP in the posterior parietal cortex (monkey X, left hemisphere; monkey S, right hemisphere). Chamber implantation was guided by anatomical MRI and stereotaxic measurements. All surgeries were performed under sterile conditions while the monkeys were under general anesthesia. The monkeys received postoperative antibiotics and analgesics. All procedures were performed in accordance with the guidelines for animal experimentation approved by authorities (Regierungspräsidium Tübingen, Germany).

3.2 Behavioral Protocol

In order to start a trial, monkeys were required to grab a bar and keep eye fixation within 1.75 degrees of visual angle of a central white dot. Then, a green square or circle background appeared on the screen during a 500 ms 'fixation period'. Subsequently, a sample stimulus consisting of a gray background containing 0 to 4 dots was shown for 500 ms. Following a 1-second-delay, during which the green background was again shown, a test stimulus appeared and the monkeys were expected to release the bar if it matched the sample stimulus in quantity. That was the case in 50% of the trials, referred to as 'match trials'. Otherwise, in non-match-trials, a 300 ms second delay was followed by a second test stimulus (500 ms) which always matched sample number. The green fixation and delay background displays framed the sample. This background was chosen to match in luminance with the gray level displayed in the trial sample stimulus. Match and non-match trials were pseudo-randomly intermixed. Correct responses were rewarded with water. Eye position was monitored with an infrared eye tracking system (ISCAN, 120 Hz sampling rate). CORTEX software (NIH, Bethesda, MD) was used in task implementation and behavioral data acquisition.

3.3 Stimuli specifications

Stimuli were shown on an LCD screen 57 cm in front of the monkey's eyes. Numerosity stimuli consisting of multiple-dot patterns against a gray background (diameter of 5.7° visual angle) were created using custom-written MatLab software. These routines enabled the generation of new stimuli sets for each session. To prevent the monkeys from memorizing the visual patterns of the displays, each quantity was tested with different images per session, and the sample and test

displays were never identical in one trial. Additionally, all conditions were shown in pseudo-randomized order in each session.

In standard trials parameters as total dot area, dot density, total stimulus luminance and background-dot ratio gradually increase or decrease with the number of dots in the stimuli, while average dot size is constant. Conversely, in control trials total dot area, dot density, total stimulus luminance and background-dot ratio are kept constant across countable numerosities (1-4) and differ drastically from empty sets, drawing a step-like function (**Fig. 2.4**). However, in control trials individual dot size decreases with numerosity (1-4).

As previously explained, in order to test how invariant is the neural representation of empty sets to image-like features we included other variants across trials. First, in each trial the background could be either a circle or a square. Second, we included two gross luminance levels (high and low) for the stimulus background. To keep the number of conditions manageable we collapsed this variant with protocol. So, control trials always exhibited a darker background (average 9.40 cd/m²) than standard trials (average 28.6 cd/m²). Again, we would expect a neural representation of zero not to vary with background luminance level (dark or light).

For behavioral reasons we introduced, in each of these 'protocol' groups, 2 sublevels of background luminance which could appear in the same trial. This strategy prevented monkeys from solving the task by identifying empty sets as those with a slightly higher total stimulus luminance (no black dots). Additionally, we could test the capacity of monkeys to match empty sets independently of how they look, darker or lighter.

3.4 Neurophysiological Recordings

In each session, arrays of up to eight glass-coated tungsten microelectrodes (Alpha Omega LTD, Israel) were inserted in each recording chamber using a grid (Crist Instruments, USA) with 1-mm spacing. Neurons were selected at random, as no attempt was made to preselect neurons according to response properties. Only stable and well isolated neurons were recorded. A MAP Plexon system was used for signal acquisition, amplification, filtering and digitalization. Waveform separation was performed off-line (Plexon Systems, USA).

3.5 Analytical Methods

Behavioral data analysis. For each session, behavioral performance functions were obtained based on the percent correct responses to all possible stimulus combinations. The overall performance tuning function was derived by averaging behavioral tuning functions over sessions.

Neuronal data analysis. Neurons were required to satisfy two criteria to be considered for further analysis: first, a minimum average firing rate of 1 Hz in the period from the start of fixation to the end of the first delay; second, at least 3 stimulus repetitions per specific condition (20 specific conditions from 5 sample numerosities x 2 types of protocol x 2 background shapes). A total of 861 parietal neurons and 476 prefrontal neurons fulfilled these criteria.

Epoch defined numerosity selectivity and tuning curves. Neuronal activity in response to numerosities during the sample phase was derived from a 500 ms interval following stimulus onset. To account for average response latencies in the respective recording areas, the analysis window was shifted by 50 ms after sample onset for VIP and 100 ms for PFC neurons. Selectivity during the delay period was evaluated in a 900 ms window starting 200 ms after the sample stimulus disappears and 100 ms after the test stimulus appears. To determine numerosity-selectivity of individual neurons, we run a 3-way ANOVA with factors number (5 sample numerosities), protocol (standard and control) and shape (circle and square). Significance was evaluated for each factor at $p < 0.01$. To create neuronal filter functions, activity rates were normalized by setting, for each neuron, the firing rate to the most preferred numerosity as 1 and to the least preferred numerosity as 0. The normalized individual tuning curves were then averaged across neurons with the same preferred numerosity.

Error trial analysis. For comparing firing rates in error and correct trials we included selective neurons with at least three non-correct trials per stimulus. We used a Wilcoxon-signed-rank test to evaluate differences between neuronal firing rates in error and correct trials.

Quantitative Index (QI). For each neuron the quantitative index was calculated as:

$$1 - \frac{AUROC_{0-1}}{AUROC_{most-least\ preferred}}$$

The denominator in the AUROC ratio normalizes QI with respect to the maximal discriminability of each neuron and guarantees unbiased comparisons between the two cortical areas.

Gaussian-Process Factor Analysis (GPFA). GPFA extracts smooth, low dimensional neuronal trajectories from the noisy spiking activity of hundreds of neurons on individual trials. While other techniques perform smoothing over time as a first step and dimensionality reduction as a second, GPFA combines these operations in one common probabilistic framework. The method was implemented using MATLAB toolboxes (Yu et al. 2009). To include a comparable number of pseudosimultaneously recorded neurons in both cortical areas and guarantee robust results, we required neurons to be recorded during a certain number of correct trials per stimulus type. We considered prefrontal neurons with at least 30 trials per numerosity (364 neurons) and parietal neurons with at least 37 trials per numerosity (377 neurons). Firing rates in

correct trials were processed using five latent dimensions and a bin width of 50 ms. Correspondent figures show average trajectories obtained after sorting trial trajectories according to sample numerosity. Trajectories are depicted in the space defined by the top 3 (orthonormalized) dimensions, sorted according to the data covariance they explain. The same analysis was repeated for exclusive selective neurons.

SVM Multiclass Classifier. In each cortical area, we created a pseudo-population of 200 neurons from the whole population. For this purpose, we considered neurons recorded by at least 20 repetitions per stimulus class. From those neurons, we selected 40 neurons preferring each sample numerosity, according to a SNR measure. This measure was calculated in the sample period as the ratio between modulation depth and variability within numerosity classes. For each neuron, we obtained firing rates from spike data using a sliding window (150 ms bin, 50 ms step). We used a multi-class linear support vector machine (SVM) classifier (Chang and Lin 2011). The ‘one-versus-one’ classification method was deployed to deal with our five classes (0-4). Ten-fold cross-validation was performed using the ‘leave-one-group-out’ paradigm. The firing rates of each neuron were normalized by z-scoring within each cross-validation repetition; normalization parameters were obtained only from the training trials. The whole procedure was repeated 50 times, each time selecting a random set of trials for each condition and then creating a new set of cross-validation splits. We report the mean performance and standard deviation over the 50 resamples. For the generalization analysis, we run the classification procedure in both directions of training and testing (for example, from circles to squares and *vice versa*). Given that no important asymmetries were found, we report the average in the main text.

Size effect Index. At the population level, we quantified how graded or categorical is the scaling of the *size effect* in both cortical areas. For that purpose, we devised a parameter that considers the magnitude of this effect between numerosity one and either empty sets and countable numerosities. Specifically, the parameter is calculated by dividing the difference between the discriminability for one and (the average discriminability for) larger numerosities, by the difference between the discriminability for empty sets and one:

$$\frac{(AUROC_{1-others}) - \sum_{c=2}^4 \frac{AUROC_{c-others}}{3}}{(AUROC_{0-others}) - (AUROC_{1-others})}$$

Time-defined ω^2 and definition of responses. To assess the influence of different factors on trial firing rates we used ω^2 PEV as measure. This parameter reflects how much of the firing rate variance across trials can be explained by different stimulus factors. ω^2 was calculated for each neuron in a sliding window (200 ms kernel, 20 ms step) from a 3-way ANOVA with main factors number, protocol and shape. Twenty five balanced permutations were run and the mean was taken as reference value. To

assess the significance of actual values, we shuffled trial labels and calculated the time defined ω^2 1000 times. For each neuron and factor, a time bin was considered significant if the probability that its actual ω^2 value is caused by chance is 1% or less, taking the 1000 shuffled corresponding values as reference (permutation test). A '*numerosity selective response*' was defined as at least 3 consecutive bins (encompassing a minimum of 240 ms) which were significant for main factor number. The stringency of our criteria was confirmed by the low incidence of numerosity selective responses, so defined, during fixation in both prefrontal and parietal neurons (<1% of responses in the sample period, with the same duration).

Selectivity Latencies. Selectivity latencies were determined by calculating ω^2 with a high temporal resolution (50 ms kernel, 1 ms shift). In each neuron the selectivity latency was defined by the first of 25 continuous bins with a significant ω^2 for number, according to a permutation test (1000 shuffled permutations, $p < 0.05$).

Response Latencies. For each neuron, we obtained firing rates in sliding 10 ms windows stepped by 1 ms. Visual latencies were determined by the first of 5 consecutive bins after sample presentation in which firing rates diverged by at least 2 standard deviations from the baseline level (taken as the middle fixation period). In each neuron, we sorted trials according to the type of sample stimulus and calculated response latencies.

Characterization of tuning profile. Following Engel (2015, for different purposes) we fitted the tuning curve of each empty set neuron with a generalized linear model (GLM) that contained a linear combination of two regressor functions: a decreasing linear function (graded tuning) and a step-like function (binary tuning). We defined the best parameters of such functions for each tuning curve by fitting. Then, we applied the GLM and obtained β coefficients. To determine whether the resulting β coefficients were significantly different from zero, we used a *t*-test to compare β against the distribution of shuffled β values, which was obtained by randomizing the trial order and then refitting the linear regression model (1,000 reshuffles). The tuning profile of neurons was classified as graded (quantitative), categorical (binary) or mixed, guided by the GLM coefficients. Each neuron was then classified as direction-tuned or category-tuned if the corresponding β was significantly different from zero ($P < 0.05$), mixed direction- and category-tuned if both β 's were significantly different from zero and nonselective if neither β was significantly different from zero.

4. Do monkeys treat empty sets as endowed with null quantity?

Matching empty sets does not demand a quantitative treatment of the stimuli. For example, the capacity to distinguish the absence and presence of countable items would suffice to succeed in trials involving empty sets. However, if monkeys treat empty sets according to the quantitative value (zero) they convey, behavior should display two hallmarks of numerical cognition: the *distance* and *magnitude effects*. The presence of these effects would amount to evidence that monkeys not only can discriminate empty sets from other stimuli, but attribute them a quantitative value in relation to countable numerosities.

4.1 Task Performance

Empty sets were introduced in the stimulus pool after monkeys had reached a stable performance with sets of 1 to 4 dots. A total of 49 behavioral sessions for monkey X and 54 for monkey S were analyzed. Behavioral tuning curves (**Figure 4.1**) show how often animals judged test stimuli as equal in quantity to each sample numerosity. Curve peaks depict the percentage of correct match trials, while non-peak values correspond to errors in non-match trials.

Notice that increasing the frequency of errors would turn the curves wider. In fact, the width of these curves reflects how accurately a sample numerosity is represented. As it is harder to discriminate large numerosities (*'magnitude effect'*), curves become wider (i.e. less selective) with increasing sample number. This translates into an increasing sigma when the curves per session are fitted with Gaussian functions on a linear numerical scale (**Figure 4.3 A,C,E,G**).

As expected if empty sets were attached a low quantitative value, the sigma of their function (red dot in **Figure 4.3 E,G**) is either smaller or similar to that of the smallest countable numerosity, one (X: 0.295 ± 0.022 for empty sets versus 0.355 ± 0.013 for 1, $p < 0.01$; S: 0.452 ± 0.018 for empty sets vs. 0.469 ± 0.015 for 1, $p > 0.05$, Mann-Whitney U-test). Moreover, the sigma of the empty set curve is in any case significantly smaller than the sigma for larger countable numerosities (2 to 4) (X: 0.295 ± 0.022 versus 0.503 ± 0.015 (sample 2, $p < 0.001$), 0.907 ± 0.014 (sample 3, $p < 0.001$), 0.888 ± 0.015 (sample 4, $p < 0.001$); S: 0.454 ± 0.018 versus 0.613 ± 0.017 (sample 2, $p < 0.001$), 1.006 ± 0.011 (sample 3, $p < 0.001$), 1.078 ± 0.027 (sample 4, $p < 0.001$), Mann-Whitney U-test). The presence of a behavioral *'size effect'*, situates empty sets in the small extreme of countable numerosities (1-4).

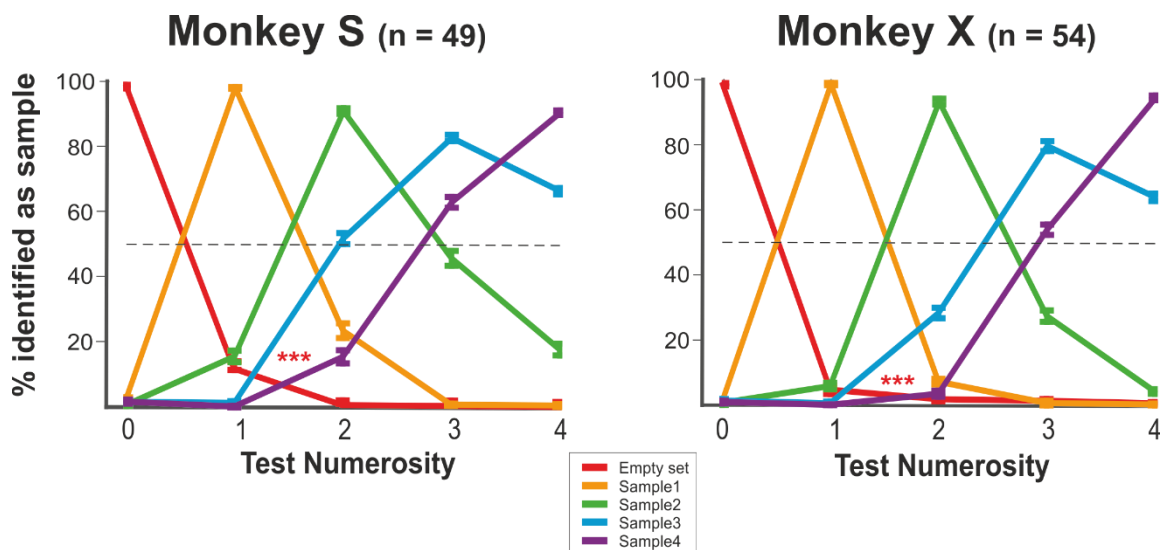


Figure 4.1 Behavioral Performance. Behavioral tuning curves derived from the monkeys' performance when different stimuli were presented as sample (0-4). The functions reflect the probability that a monkey judged displays in the test period as containing the same number of items as the sample numerosity (indicated in various colors). The peak data point of each colored curve indicates the correct performance in match trials for the different sample numerosities. Data points to the left and right of the peak reflect performance in non-match trials (i.e. when the first test numerosity was smaller or larger than the sample).

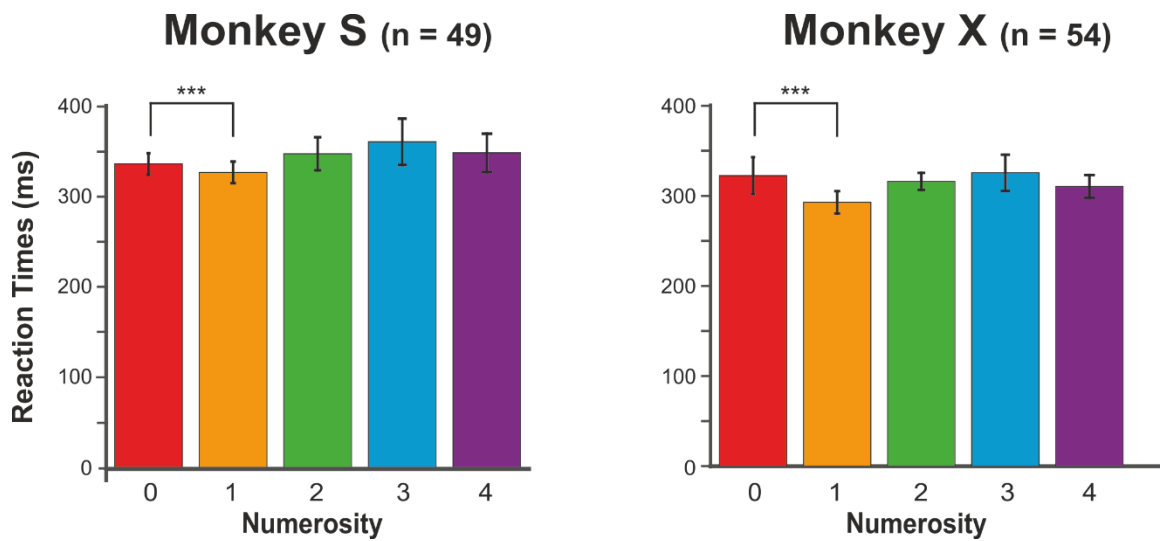


Figure 4.2 Reaction Times. Reaction times for the different stimuli in match trials. If treated as endowed with numerosity 0, empty sets should elicit shorter reaction times than numerosity 1. However, reaction times in empty set trials are longer than expected in both subjects.

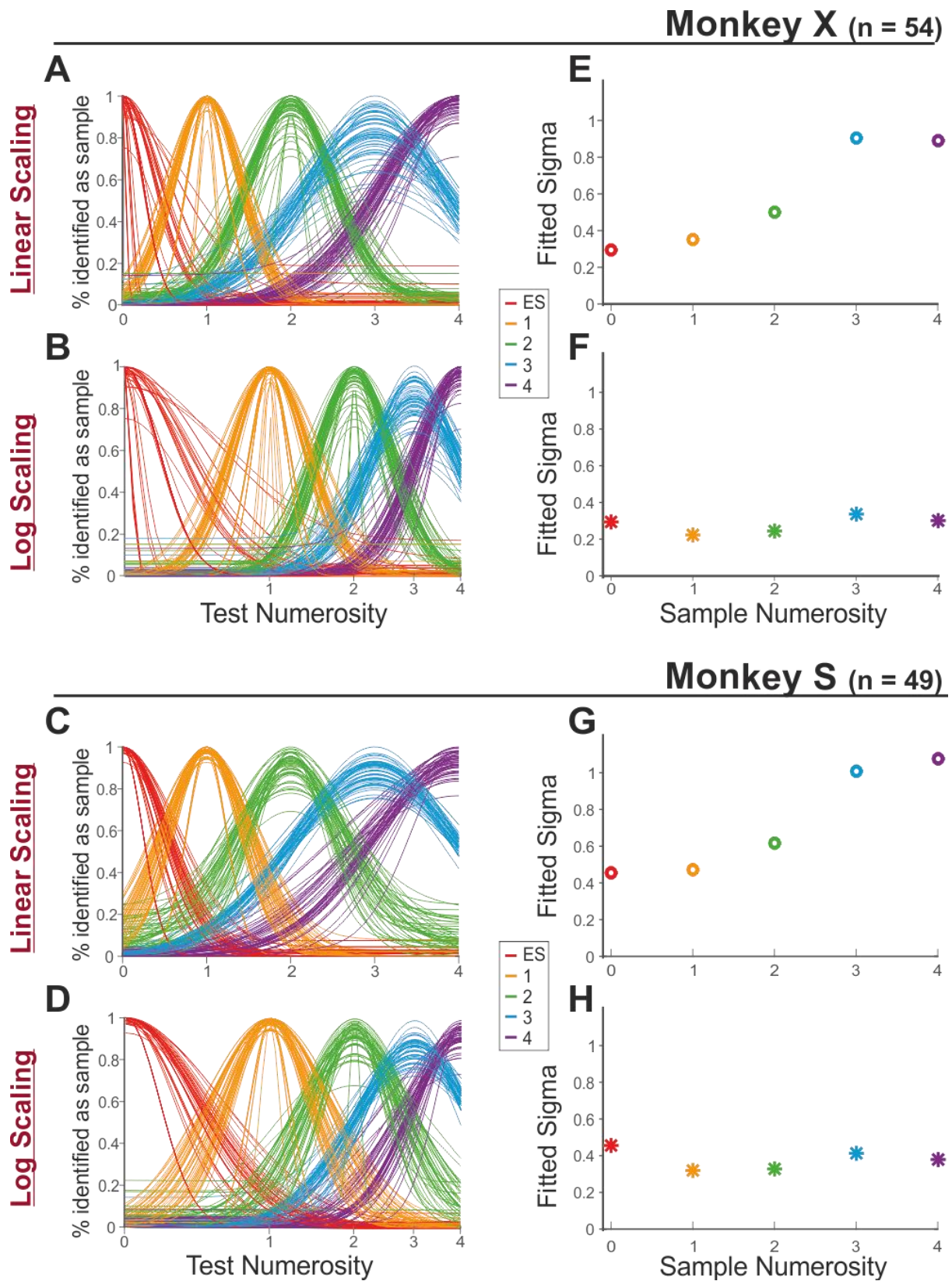


Figure 4.3 Behavioral Fittings. Tuning curves per session were fitted with a Gaussian function in a linear (A,C) and a logarithmic (B,D) numerosity scaling. In a linear scaling, fitted sigmas tend to increase with sample numerosity (E,G). In a logarithmic scaling, (F,H) fitted sigmas become similar for the different sample numerosities.

A non-linearly compressed number line could account for the increase in sigma for larger numerosities. We explored whether behavioral performance curves could be better described on a linear or nonlinearly compressed number line. For that purpose, we now fitted the behavioral curves with a Gaussian function when plotted on a logarithmic ($\log_2(n+1)$) numerical scale (**Figure 4.3 B,D**). The goodness-of-fit (r^2)-values were taken as a measure of which scaling scheme describes better the data. For each monkey, the fittings were significantly better on a logarithmically compressed number line (both monkeys $r^2=0.98$) than on a linear scale (versus $r^2=0.94$ in monkey S and $r^2=0.95$ in monkey X, $p<0.001$ in both subjects; Wilcoxon sign rank test). Thus, as predicted by the Weber-Fechner law, skewed behavioral curves became more symmetric when plotted on a logarithmic scale. Moreover, as previously described, on a logarithmic scale performance curves exhibit similar sigmas for the different numerosities (**Figure 4.3 F,H**).

Finally, since empty sets are represented very precisely (small sigmas), monkeys made few mistakes in trials in which they appeared. However, the distribution of those errors offers further insight into how empty sets are ordered with respect to countable numerosities. When empty sets were presented as sample (red line in **Figure 4.1**), monkeys mistakenly matched them to numerosity 1 more frequently than to numerosity 2 (Monkey S: $12.48\pm 1.31\%$ vs. $1.27\pm 0.41\%$, $p<0.001$; Monkey X: $4.82\pm 1.32\%$ vs. $2.02\pm 0.69\%$, $p=0.0011$, Wilcoxon signed rank test). This pattern in behavior, a '*numerical distance-effect*', suggests empty sets were situated closer to 1 than to 2 in the number line.

4.2 Reaction Times

Altogether, the presence of distance and magnitude effects in performance suggests empty sets were properly positioned in the numerical continuum and so treated in a quantitative way. However, such treatment demanded more time than expected in the case of empty sets. Given that larger numerosities are represented less precisely, reaction times tend to increase with numerosity. We can see this pattern in our data with numerosities ranging from 1 to 3. For numerosity 4 short reaction times can be explained by an 'edge effect': the border elements in the range of stimuli are easier to discriminate.

All these considerations taken into account, one would expect that, if zero finds a place in our visual sense of number, it triggers very fast responses. But reaction times are longer in match trials involving empty sets than in those involving numerosity one (Monkey S: 336.28 ± 1.69 versus 326.89 ± 1.70 ms, $p<0.001$; Monkey X: 322.47 ± 2.79 versus 292.86 ± 1.68 ms, $p<0.001$, Wilcoxon signed rank test) (**Figure 4.2**). This pattern prevails when the different conditions are separately analyzed (**Figure S1**). Additionally, this is the case even long after the introduction of empty

sets in the stimuli pool (**Figure S1**), and thus this effect cannot be explained by stimulus novelty.

5. Is zero represented in the primate visual sense of number?

In this first part of the project, our main aim was to explore whether zero is encoded in the primate visual sense of number. Therefore, we focused our analysis in the trial epoch when stimuli are available to the monkey: the **sample period**. In total, 861 VIP neurons (431 from monkey X and 430 cells from monkey S) and 476 prefrontal neurons (279 neurons from monkey X and 197 cells from monkey S) satisfied the basic criteria to be further considered in the initial analysis of the sample epoch (*See Methods for details*).

5.1 Single-neuron representation of empty sets

During the **sample period** an important proportion of neurons were strongly modulated by sample numerosity, as their firing rates reflected the numerical distance between stimuli. **Figure 5.1** shows three example neurons from VIP (**Figures 5.1 A-C**) and PFC (**Figures 5.1 D-F**). As with countable numerosities, many neurons discharged maximally to empty sets (empty sets as ‘preferred numerosity’) (**Figure 5.5 A,D**) or responded least to them (‘least preferred numerosity’) (**Figures 5.1 B,C,E,F**).

Table 5.1 shows the proportions of cells which, during the sample period, were selective for the different main factors and interactions between main factors. Neurons which only showed a significant main effect for factor ‘number’ and no significance for any other main factor or factor interactions were identified as ‘*exclusive number selective neurons*’. In the sample period, 8% (70/861) VIP neurons and 16% (78/476) PFC neurons belonged to this most conservatively determined subpopulation.

ANOVA Factor	VIP (n=861)	PFC (n=476)
Number	18.9%	38.9%
<i>Excl. Number</i>	8.1%	16.4%
Protocol	8.8%	12%
Shape	17.7%	18.9%
Number-Protocol	3.7%	5.5%
Number-Shape	4.6%	10.9%
Protocol-Shape	2.7%	3.8%

Table 5.1
Neuronal selectivity for the different task factors in the sample period. Calculated with 3-factor ANOVA on sample trial firing rates and evaluated at $p < 0.01$.

Hereafter, the terms ‘factor number’, ‘factor protocol’ and ‘factor shape’ are used to refer to the different conditions. Nonetheless, ‘factor protocol’ incorporates not only dot density and total dot area, but also background luminance and contrast parameters. Additionally, ‘factor number’ includes empty sets and stimuli with 1 to 4 dots. However, we do not assume empty sets are neurally represented as a numerosity.

We identified the preferred numerosity of each selective response, defined as the stimulus that elicited the highest average activity. **Figures 5.2 B,D** show the distribution of preferred numerosities in VIP and PFC during the sample period. In prior studies, not including empty sets, numerosity one, the smallest numerosity in the stimulus pool, is preferred with the highest frequency. Accordingly, in both cortical areas and both epochs empty sets are the most frequently preferred stimulus, both in VIP (**Figure 5.2B**) and PFC (**Figure 5.2D**).

We constructed population tuning functions of exclusive selective neurons by normalizing the firing rates of individual neurons to the different sample stimuli, and then averaging those with the same preferred numerosity (*See Methods for details*). The tuning curves of neurons preferring countable numerosities 1 to 4 in VIP (**Fig. 5.2A**) and PFC (**Fig. 5.2C**) showed a clear distance effect, i.e. a progressive drop-off of activity with increasing numerical distance from the preferred numerosity.

Next, we investigated which scaling scheme (linear or logarithmic) accounted better for the neuronal data. In VIP, a logarithmic scaling did not result in better fittings of selective neurons’ tuning functions ($r^2=0.79$ for both scaling schemes, $p=0.83$, Wilcoxon sign rank test, $n=70$). In numerosity-selective PFC neurons, however, the goodness-of-fit values were significantly higher in a logarithmic scaling ($r^2=0.82$) than in a linear scaling ($r^2=0.80$) ($p<0.05$, Wilcoxon sign rank test, $n=78$). This indicates that, in agreement with the behavioral data, numerical magnitudes in PFC are best represented on a non-linearly compressed scale.

5.2 VIP neurons represent empty sets as a different stimulus from countable numerosities

At this point a crucial clarification needs to be done: That a neuron fires maximally to empty sets does not mean it represents numerosity zero. We would expect zero-tuned neurons to show not only maximal activity for empty sets, but also a *distance effect* in its firing rates. Particularly, zero neurons should fire more to numerosity one than to other countable numbers, as the former is closer to zero in the numerical continuum. **Figures 5.2 A,C** show the average tuning curves of empty-set preferring neurons in red, respectively in VIP and PFC. Note that VIP empty set

neurons respond to countable numerosities with similar levels of activity (**Fig. 5.2A**) (0.318 ± 0.05 for 1, 0.233 ± 0.037 for 2, 0.181 ± 0.02 for 3, 0.166 ± 0.045 for 4, $n=28$). For example, the response to 1 does not differ from the response to numerosity 2 (0.318 ± 0.05 for 1 vs. 0.233 ± 0.037 for 2, $p=0.096$, $n=28$). Then, these parietal neurons seem to discriminate empty sets from all countable numerosities in a more binary fashion, drawing a step-like function (**Fig. 5.3A**). This type of tuning could reflect a categorical representation, in which empty sets are represented as different from all countable numerosities.

Differently, the average tuning curve of PFC empty set tuned neurons (**Fig. 5.2C**, solid red line) exhibits a progressive decline in firing rates with increasing numerosity (0.463 ± 0.06 for 1, 0.229 ± 0.033 for 2, 0.116 ± 0.025 for 3, 0.043 ± 0.016 for 4, $n=24$). Particularly, prefrontal neurons responding maximally to empty sets fired more to numerosity 1 than to numerosity 2 ($p < 0.001$, $n=24$; Wilcoxon signed rank test). Subsequently, prefrontal empty set neurons also responded more to numerosity 2 than to numerosity 3 (0.12 ± 0.025) ($p < 0.01$). Such pattern of response entails a 'distance effect' for empty sets and is characteristic of numerical representations.

Finally, note that in a binary tuning profile numerosity 1 is represented farther from empty sets and closer to other numbers (**Fig. 5.3A**). Comparatively, in graded responses, numerosity 1 lies closer to empty sets and farther from larger countable numbers (**Fig. 5.3B**). We devised an index to measure how graded or binary is the neuronal tuning for empty sets. This '**quantitative index (QI)**' uses ROC analysis to evaluate the discriminability between empty sets and numerosity 1, relative to the maximal discrimination capacity of a given neuron (*See Methods for details*). For clarity purposes, lower QI values indicate a more binary tuning profile, while high QI values correspond to a more graded tuning.

$$QI = 1 - \frac{AUROC_{0-1}}{AUROC_{most-least}}$$

The denominator in the AUROC ratio normalizes QI with respect to the maximal discriminability of each neuron and guarantees unbiased comparisons between the two cortical areas. Binary tuning profiles correspond to lower QI values, while the graded tuning characterizing number selectivity will be marked by high values.

Empty set neurons in VIP exhibit a lower QI value than prefrontal empty set neurons (0.071 ± 0.016 in VIP versus 0.155 ± 0.023 in PFC, $p < 0.01$, Mann-Whitney U-test). Importantly, that was also the case for all exclusive number neurons, irrespective of their preference (0.114 ± 0.011 in VIP versus 0.154 ± 0.013 in PFC, $p < 0.05$, Mann-Whitney U-test) during the sample period.

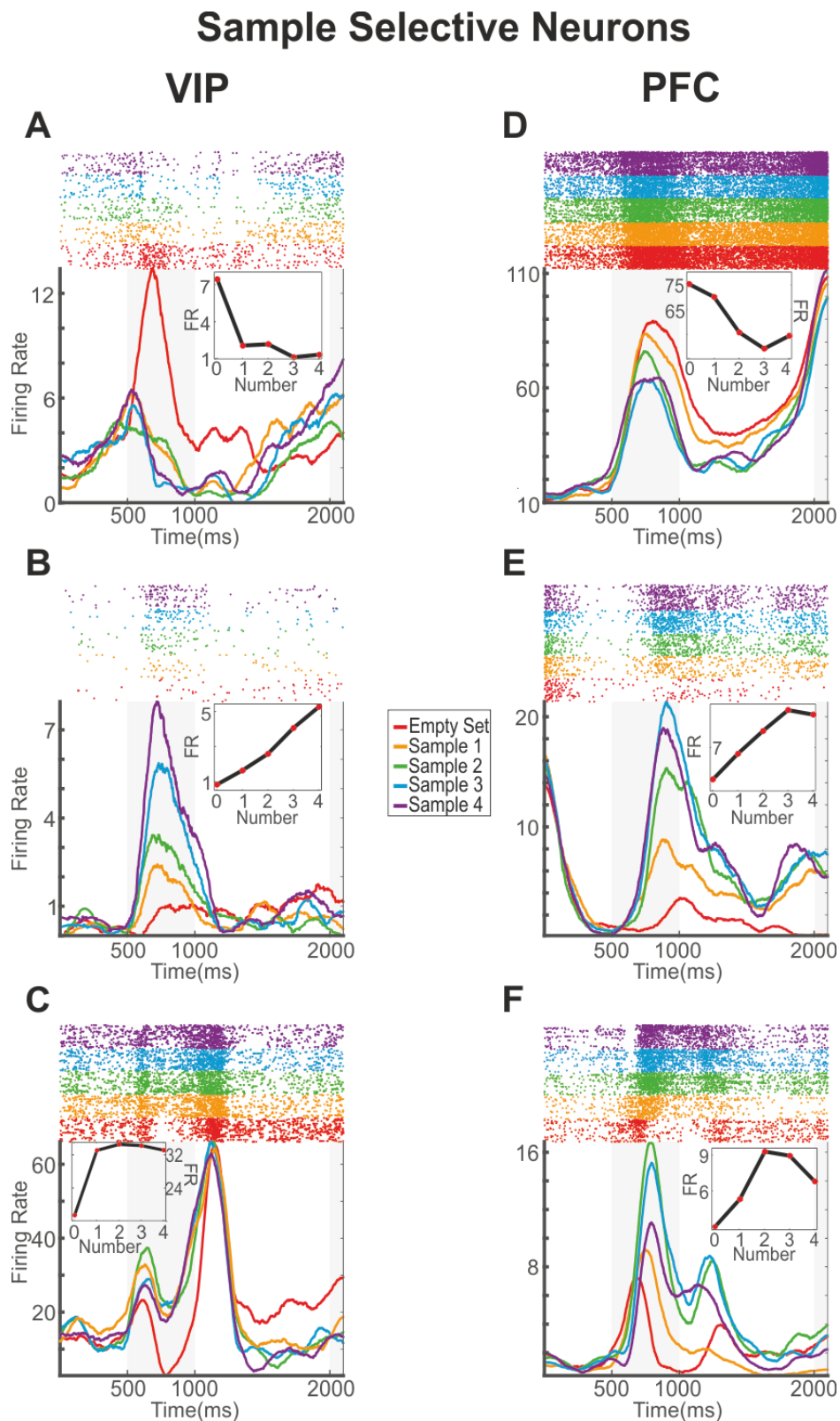


Figure 5.1 Neurons with numerosity selective responses during the sample period. (A-C) Example numerosity selective neurons in VIP. Top panel shows dot-raster histograms (each dot represents an action potential); bottom panel depicts averaged spike density functions (activity averaged in a sliding 150 ms window). The first 500 ms represent the fixation period, followed by the sample and delay periods. Inset in spike density plot shows the neuron's tuning function (i.e., discharge rates as a function of the number of presented items) during the grey shaded sample period. (D-F) Example numerosity selective neurons in PFC.

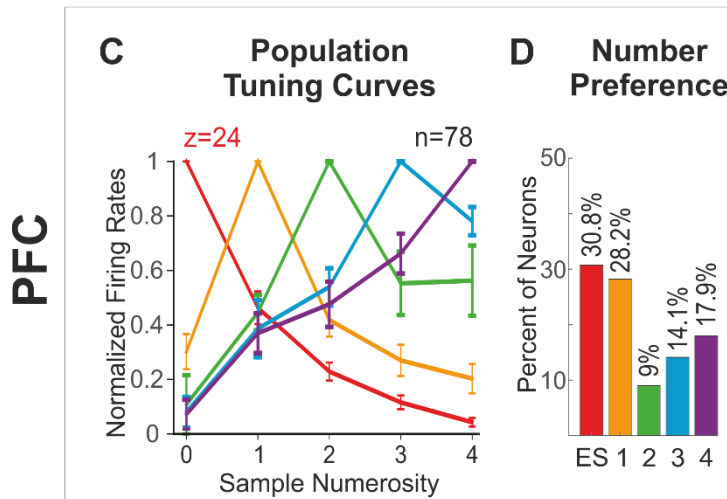
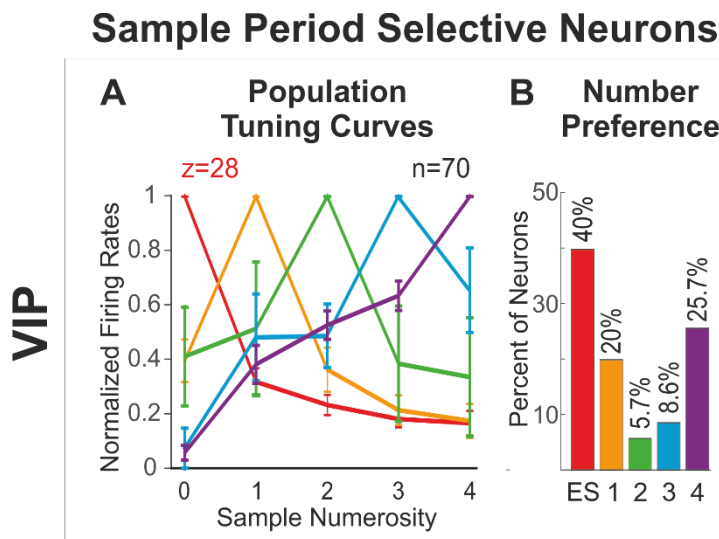


Figure 5.2 (A,C) Population tuning curves obtained by averaging the normalized tuning curves of VIP and PFC neurons with the same preferred stimulus. The average tuning curve of empty set preferring neurons is shown in red. **(B,D)** Proportion of neurons in VIP and PFC responding maximally to each of the stimulus types.

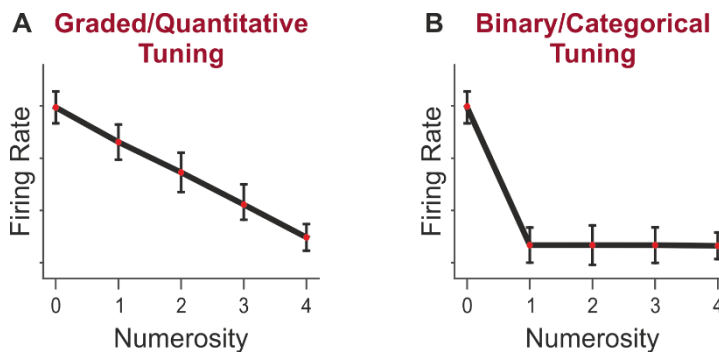


Figure 5.3 Empty-set preferring neurons. Schematic representation of two types of tuning in neurons responding maximally to empty sets.

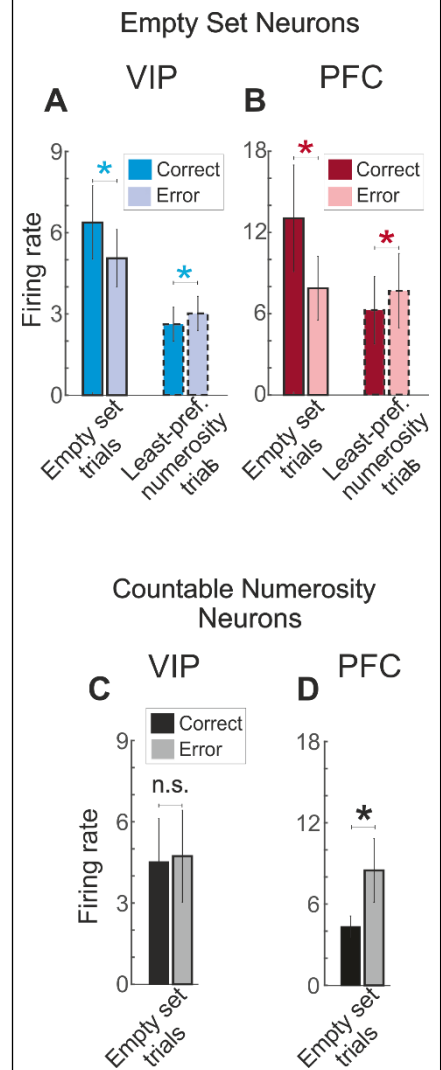


Figure 5.4 Error Trial Analysis. (A& B) Behavioral Relevance of empty-set preferring neurons in empty set trials (preferred stimulus) and countable numerosity trials (least-preferred stimulus). The neuronal firing rates in the sample period are compared between correct and error trials. **(C&D)** Firing rates of VIP and PFC countable-numerosity preferring neurons in correct and erroneous empty-set trials. Error bars indicate the SEM.

5.3 Behavioral relevance of selective-neurons' activity during the sample period

To further investigate to what extent the activity of exclusive numerosity selective neurons in either VIP or PFC is related to behavioral performance, we compared their activity in error and correct trials. First, we explored whether the firing rate of empty-set tuned neurons correlated with successful completion of the task. If the responses of these neurons to empty sets (their preferred stimulus, i.e. eliciting maximal responses) were relevant for trial outcomes, lower firing rates would be expected in failed empty set trials. Indeed, the responses of VIP (**Fig. 5.4A**) and PFC empty-set neurons (**Fig. 5.4B**) were decreased in erroneous empty set trials during the sample period (VIP: 6.33 ± 1.26 Hz versus 5.03 ± 0.10 Hz, $n=15$, $p<0.05$; PFC: 13.04 ± 3.94 Hz versus 7.58 ± 2.34 Hz, $p<0.05$, $n=10$; Wilcoxon signed-rank test). This result suggests that the activity of empty set neurons in both VIP and PFC is relevant for the outcome of trials in which an empty set was presented as sample.

Does the activity of empty-set neurons also correlate with performance in countable numerosity trials? In correct trials, countable numerosities were encoded with low firing rates by empty set neurons. If this low activity were relevant for performance, higher firing rates to the non-preferred numerosities of empty set neurons might lead to errors. We compared the firing rates of empty set neurons to their least preferred stimulus (a countable numerosity) in correct and error trials. Empty-set neurons in VIP (**Fig. 5.4A**) and PFC (**Fig. 5.4B**) exhibited higher firing rates to their non-preferred numerosity in error compared to correct trials during the sample period (VIP: 2.61 ± 0.63 Hz versus 3.01 ± 0.63 Hz, for correct and error trials, $p<0.05$, $n=24$; Wilcoxon signed-rank test, PFC: 6.54 ± 2.5 Hz versus 7.67 ± 2.74 Hz, for correct and error trials, $p<0.05$, $n=23$; Wilcoxon signed-rank test). Thus, the activity of empty-set neurons in both cortical areas was also correlated with the outcome of trials in which countable numerosities were presented as sample.

Finally, is the activity of countable-numerosity neurons, in turn, correlated with performance in empty-set trials? In this case, we analyzed the error-trial activity of countable-numerosity neurons which fired the least for empty sets in correct trials. The activity of countable-numerosity neurons in VIP (**Fig. 5.4C**) did not differ in error and correct empty set trials (4.51 ± 1.60 versus 4.73 ± 1.7 , $p=0.86$, $n=14$). In countable-numerosity PFC neurons (**Fig. 5.4D**), however, the activity during the sample period increased in erroneous empty-set trials (4.33 ± 0.80 versus 8.49 ± 2.35 , $p=0.04$, $n=9$; Wilcoxon signed-rank test). So, only the activity of PFC countable-numerosity neurons during the sample period is correlated with the outcome of empty-set trials. Particularly, when empty sets are shown as sample, a decrease in the response of PFC number neurons is necessary for the successful completion of the trial. Only in

PFC, neurons preferring countable numerosities contribute to the representation of empty sets.

5.4 Population tuning for empty sets in VIP and PFC

Then, we explored how VIP and PFC whole neuronal populations, irrespective of selectivity status or stimulus preference, encode empty sets. In both parietal and prefrontal cortices some stimulus or task features have been found to be randomly represented across neurons (Mante et al. 2013; Raposo, Kaufman, and Churchland 2014). So, a certain parameter could be encoded in the combined activity of an arbitrary number of neurons. Therefore, at the population level empty sets could be represented in the numerical continuum even if selective single-neurons do not seem to encode them so.

To investigate this possibility, we analyzed the coding capacity and dynamics of population responses as a whole by performing a multidimensional state space analysis (Gaussian-Process Factor Analysis, GPFA) (Yu et al. 2009) on similarly sized pseudo-populations of neurons in VIP and PFC. This approach extracts trajectories from the spiking activity of a neuronal population in individual trials. Such trajectories reflect the instantaneous firing rate of the respective neuronal population as they evolve over time. **Figures 5.5 A,B** depict average population trajectories for the different sample stimuli in a space defined by the top three most meaningful dimensions. To guarantee robust results we required neurons to satisfy a signal to noise ratio criterion and include a certain number of correct trials per stimulus type (377 neurons in VIP and 364 neurons in PFC).

To evaluate the population numerical tuning, we measured Euclidian distances between trial trajectories corresponding to different samples. In VIP, parietal population dynamics did not exhibit a distance effect for empty sets (**Fig. 5.5C**). The inter-trajectory distances between empty sets and different countable numerosities (0-1, 0-2, 0-3 and 0-4) do not differ from each other (time defined Kolmogorov Smirnov permutation test, comparing the distributions of inter-trial distances for the different pairs of stimulus, 1000 random permutations, alpha level 0.05, **Fig. 5.5E**).

In contrast, in the ordered layout formed by prefrontal trajectories (**Figure 5.5B**), a population distance effect could be clearly identified. Trajectories take distance from each other after sample stimulus presentation and, notably, their positioning reflects the quantitative relations between sample numerosities. The closer two numerosities were in the numerical continuum, the more similar were their patterns of population activity, and *vice versa*. This held true for empty sets. The distance between population trajectories in empty set trials and other trials increased with the sample magnitude of the latter (**Figure 5.5D**, inset). Indeed, all inter-trajectory

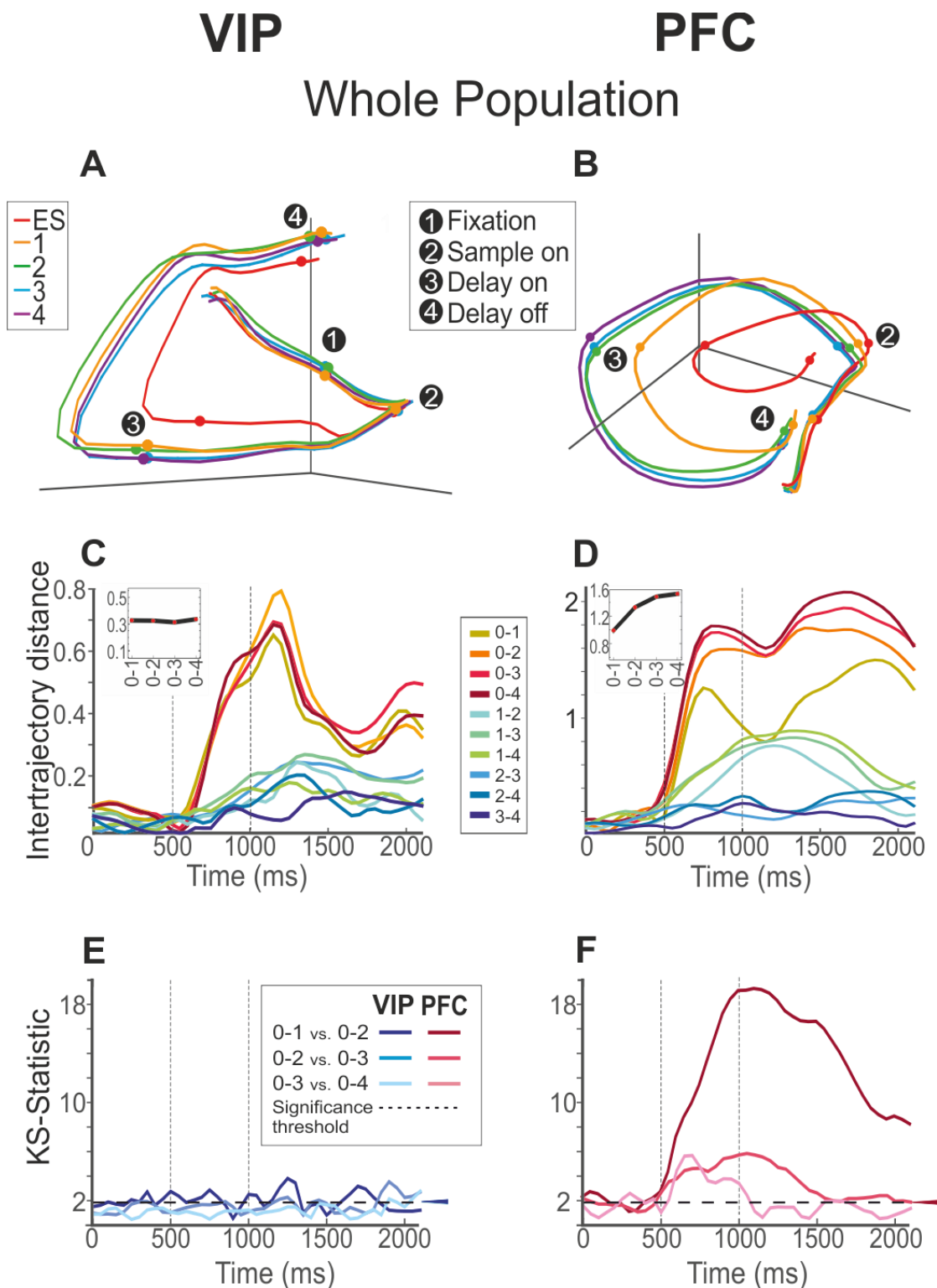


Figure 5.5 Population dynamics in VIP and PFC (A & B) Average whole population state-space trajectories in VIP (A) and PFC (B) in trials with different sample stimuli. **(C & D)** Average distances between trials with different sample numerosities in the whole population of VIP neurons (C) and PFC neurons (D). **(E & F)** Statistical comparison of pairs of inter-trajectory distances that define a distance effect for empty sets in the whole population of VIP (E) and PFC neurons (F). The distributions of trial intertrajectory distances were compared with a Kolmogorov-Smirnov permutation test per time bin. The significance threshold for each comparison, evaluated at an alpha of 0.05 is marked with a dotted line and a lateral colored arrow.

Num.Selective Neurons

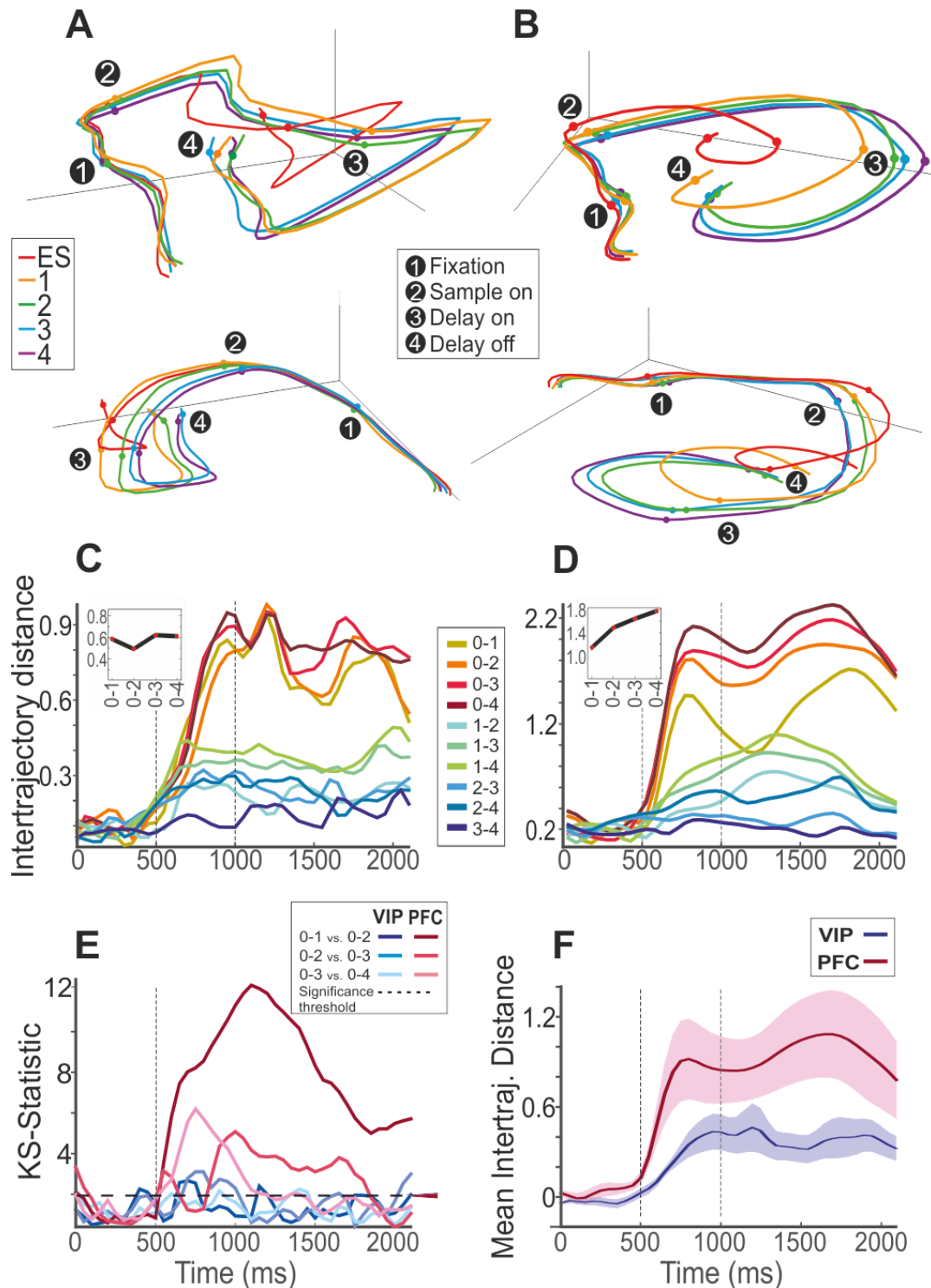


Figure 5.6 Dynamics in VIP and PFC populations of selective neurons. (A, B) Average whole population state-space trajectories in VIP (A) and PFC (B) in trials with different sample stimuli. (C, D) Average distances between trials with different sample numerosities in the population of VIP (C) and PFC selective neurons (D). (E) Statistical comparison of pairs of inter-trajectory distances that define a distance effect for empty sets in both populations. (F) Mean intertrajectory distance between all pairs of sample numerosities in VIP and PFC populations.

distance comparisons which defined a distance effect for empty sets surpassed their significant threshold in PFC (**Figure 5.5F**) during the sample period. In addition, the analysis was performed in the exclusive selective population of neurons (**Figure 5.6**). Results exhibited the same patterns: a distance effect was largely absent in VIP exclusive number selective neurons, but clearly present in PFC exclusive number selective neurons.

5.5 Decoding stimulus identity from population activity

We trained a support vector machine (SVM) classifier to discriminate numerosity on the spiking activity of either VIP or PFC neurons (**see Methods**). Preference-balanced pseudo-populations of 200 neurons were assembled per cortical area. **Figures 5.7 A&B** show the cross-training performance of the VIP and PFC classifiers, i.e. their accuracy to identify the correct numerosity when tested on the activity from a certain trial time period after being trained on another time. With a chance performance of 20% (for five classes), the classifier accuracy was higher in prefrontal than in parietal neurons throughout the sample phase (**Figure 5.7C**, VIP $50.1\% \pm 7.7\%$; PFC $67.8\% \pm 7.1\%$, mean \pm s.d. over resamples, training and testing in the same time bin).

Additionally, classification performance reflected the effects described in behavior. In general, accuracy decreased along the diagonal of the confusion matrix with increasing numerosities ('size effect'), and the probability of misclassification of trials (perpendicular to the diagonal) increased the closer two classes are in the numerical space ('distance effect') (**Figure 5.8**).

The confusion matrix for VIP (**Figure 5.8**, left) shows a robust accuracy for empty sets and numerosity 1, but only weak accuracy for other numerosities in VIP neurons (see also the resulting flat accuracy curves derived from the confusion matrix in the top left panel of **Figure 5.8**). In contrast, classification performance with PFC neurons was robust for all stimulus classes (**Fig. 5.8**, right), which was also reflected by sharp accuracy curves (**Figure 5.8**, right top panel).

Next, we evaluated the ability of the classifier to discriminate each class (sample stimulus) from all others using the area under the ROC-curve (AUROC) as measure (**Figure 5.9**; chance level 0.5). If magnitude classes were ordered along a numerical continuum, we would expect a graded decrease of discriminability with increasing numerical magnitude as a signature of the 'numerical size effect'. In VIP the average AUROC-values during the sample period were 0.94 ± 0.04 for empty sets, 0.81 ± 0.09 for numerosity 1, and 0.68 ± 0.07 for larger numerosities (mean \pm standard deviation over resamples).

Note that the classifier's capacity to discriminate numerosity 1 was similar to the discriminability of larger numerosities (**Figure 5.9**, left). This indicates only a mild size effect in VIP neurons for countable numerosities. In contrast, the classifier's discriminability function for empty sets showed higher values compared to the discriminability of all countable numerosities. This decoding pattern again suggests that empty sets are treated as categorically different from other stimuli.

In PFC, however, a clear gradation of discriminability values from empty sets to higher numerosities was present (**Figure 5.9**, right). Numerosity 1 was discriminated much better than other countable classes and slightly worse than empty sets (empty sets: 1.0 ± 0.01 , numerosity 1: 0.95 ± 0.04 , other numerosities: 0.80 ± 0.06 , mean \pm standard deviation over resamples). Note the graded decrement in the discriminability of empty sets, numerosity 1 and larger numerosities in PFC. This pattern evidences a 'numerical size effect' at the population level and provides further evidence that prefrontal neurons integrate empty sets as part of the numerosity continuum.

To quantify how graded or categorical is the scaling of the *size effect* in both cortical areas we used a parameter that considers the magnitude of this effect between countable numerosities and with respect to empty sets. Specifically, the parameter is calculated by dividing the difference between the discriminability for one and (the average discriminability for) larger numerosities, by the difference between the discriminability for empty sets and one:

$$\frac{(AUROC_{1-others}) - \frac{\sum_{c=2}^4 AUROC_{c-others}}{3}}{(AUROC_{0-others}) - (AUROC_{1-others})}$$

The higher the value of this ratio, the more graded is the scaling of the *size effect*. Analysis of the *size effect* in the classifiers' performance reveals a more graded stimuli encoding in PFC and a more categorical encoding in VIP (VIP 1.37 ± 0.26 , versus PFC 3.46 ± 0.26 , $p < 0.001$, Mann-Whitney U test).

5.6 Level of abstraction of empty set representations

So far, we excluded the putative effect of low level visual features on tuning by analyzing exclusively numerosity selective neurons. To directly address the level of abstraction of empty-set representations at the whole population level, we tested the effects of protocol and background shape with a decoding approach. We trained the SVM classifier on circle trials and tested it on square trials, and vice versa (shape generalization). We also tested generalization across protocols, with different background gray level.

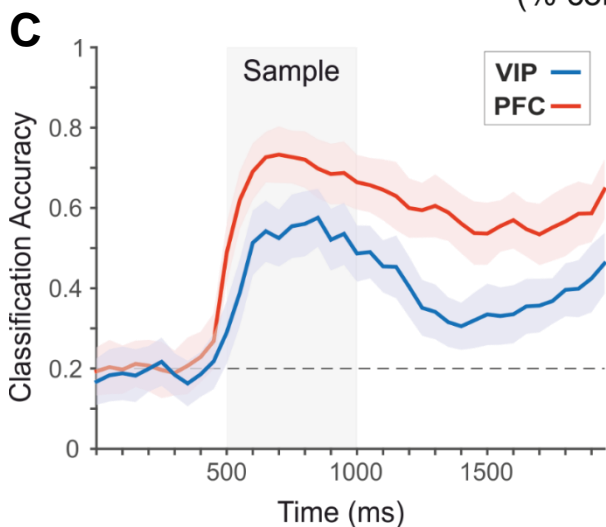
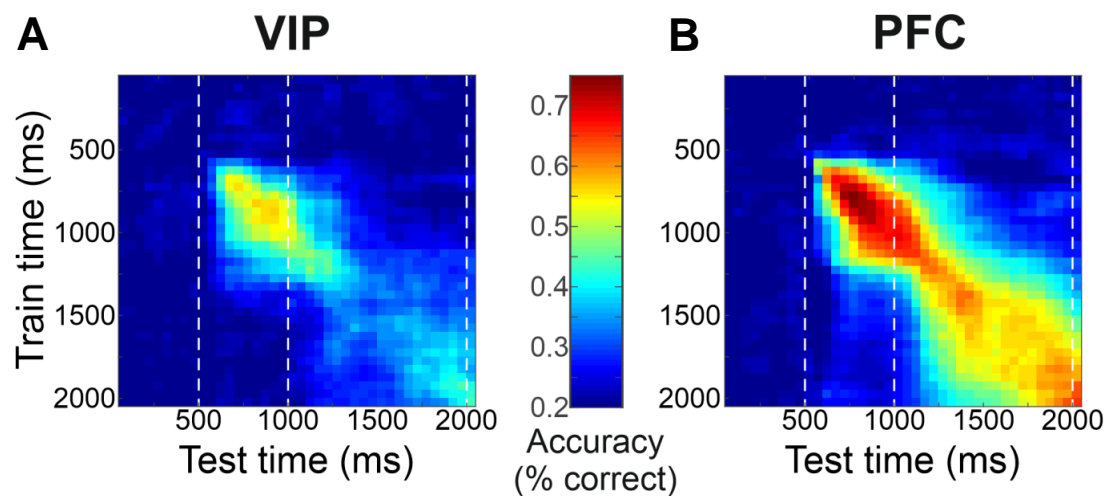
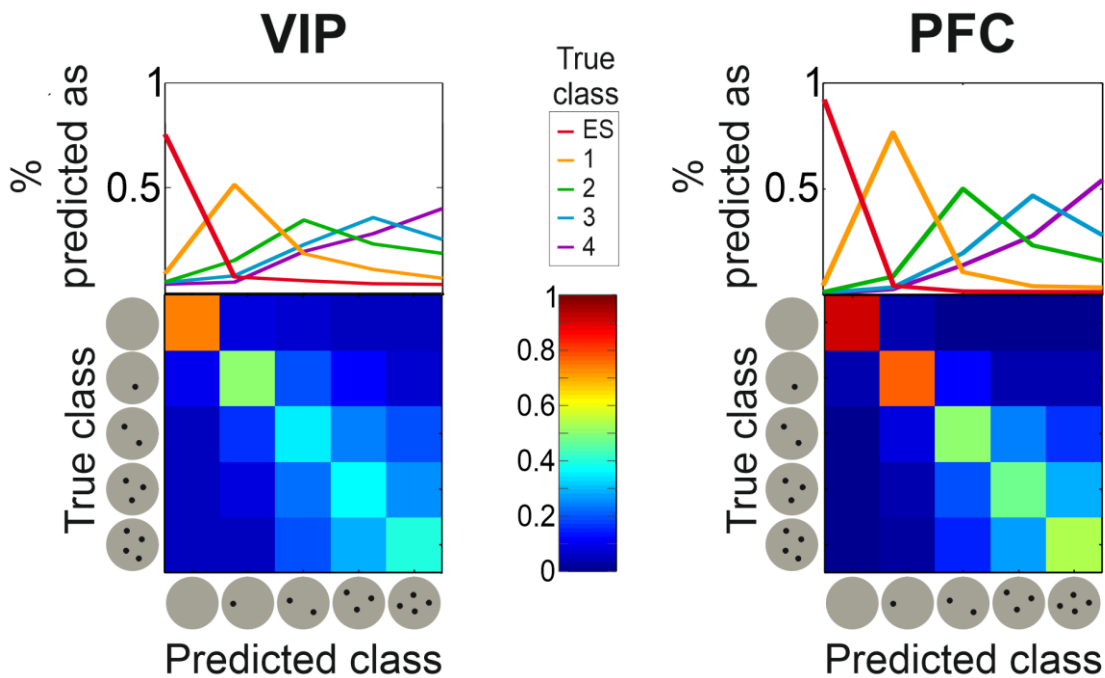


Figure 5.7 Decoding numerosity from population activity with a SVM classifier. **(A&B)** Temporal cross-training classification accuracy in VIP (left) and PFC (right) populations. **(C)** Numerosity classification accuracy in VIP (blue) and PFC (red) when training and testing are performed in the same time bin.

Figure 5.8 Decoding accuracy during the sample period for the different stimuli. Confusion matrices show the proportion of test trials in which the classifier labeled the activity elicited by a stimulus (true class) as corresponding to other stimulus type (predicted class).



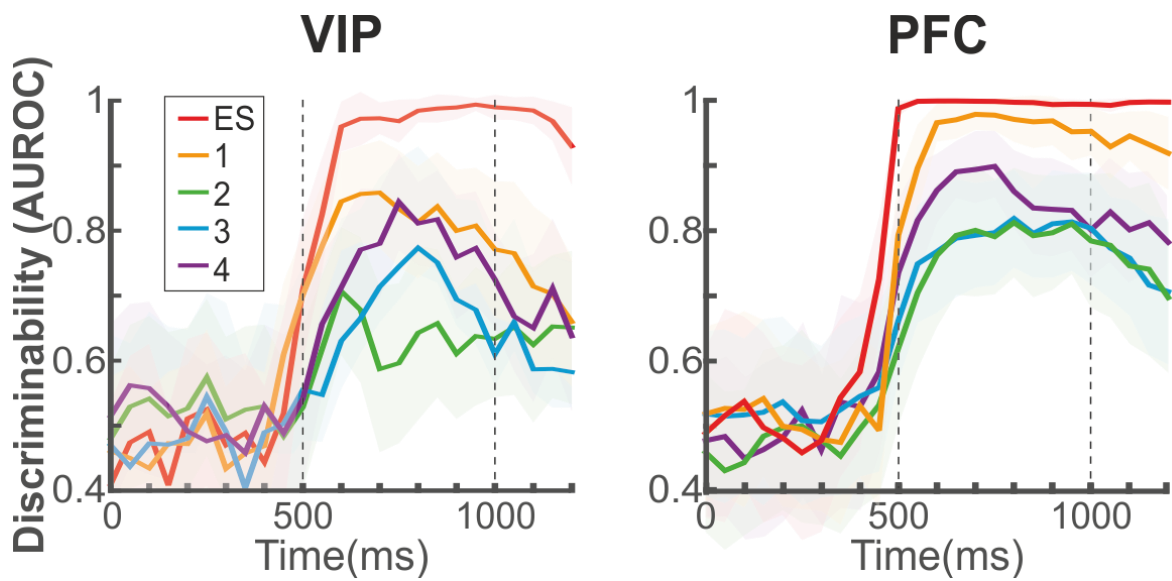


Figure 5.9 Discrimination of different sample numerosities by the SVM classifier. AUROC values were used to evaluate the discriminability of each numerosity versus the others by the SVM classifier. Numerosity was decoded from the firing activity of parietal (left) and prefrontal (right) neurons.

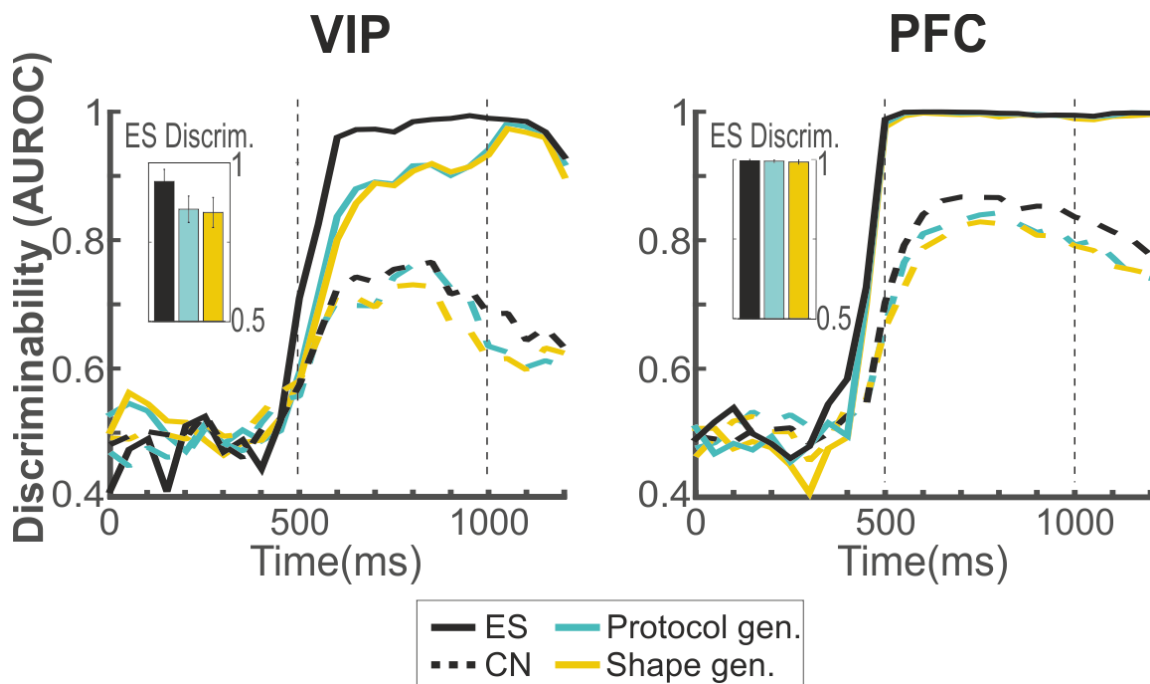


Figure 5.10 Generalization performance of the SVM classifiers in VIP and PFC. The discriminability of empty sets is depicted in solid lines, whereas dotted lines represent the discriminability of countable numerosities. Performance when training and testing were implemented in trials from different protocols (standard and control) is depicted in blue. Yellow represents generalization performance across different background shapes (circles and squares). The bars in the insets show the average discriminability of empty sets by the classifier during the sample period, when the stimulus is visually available to the subject. The performance achieved across different conditions is compared to the base performance, when both training and testing were performed on mixed datasets, including trials from all conditions. ES: Empty sets, CN: Countable numerosities.

Interestingly, the discriminability of empty sets by VIP neurons (0.94 ± 0.04 , mean \pm standard deviation over resamples) dropped by 19.7 % (0.85 ± 0.04) when training and testing were implemented in trials with different protocols (**Figure 5.10**, left, compare black and blue lines). Similarly, discriminability decreased by 22 % (0.84 ± 0.05) in shape generalization (**Figure 5.10**, left, compare black and yellow lines). Parietal representations of empty sets are thus considerably influenced by visual stimulus properties.

Classification performance based on PFC neurons, however, fully generalized across sample appearance (**Fig. 5.10**, right). The classifier's ability to discriminate empty sets (1.0 ± 0.01) was not affected by training and testing on trials from different conditions. Discriminability decreased by only 0.2 % in protocol generalizations, and by 0.8% in shape generalization (**Fig. 5.10**, right, compare black line to blue and yellow lines, respectively). Thus, PFC representation of empty sets is invariant to background shape and gray level. This result points to a more abstract representation of empty sets in PFC, detached from stimulus appearance and low level properties.

5.7 Conclusions

We assessed the neuronal representation of empty sets in two monkeys trained to perform a delayed match-to-sample task using empty sets and countable numerosities as stimuli. Behavioral performance confirmed that monkeys assess empty sets according to the null quantity they convey. Simultaneous recordings from VIP and PFC showed that, as for countable numerosities (1-4), a proportion of recorded neurons are tuned to empty sets.

A differential tuning for empty sets emerged between the two cortical areas. Parietal empty set neurons responded similarly to different countable numerosities, failing to exhibit a neuronal distance effect. The absence of a progressive drop-off of activity with numerical distance disqualifies the characterization of these neurons as numerosity tuned. The resulting binary tuning profile can be better described as signaling the presence or absence of countable items. Our population analysis argues that VIP does not genuinely represent empty sets as part of the numerosity continuum. Rather, empty sets were encoded as a separate category, different from all other numerosities. The population state space analysis confirmed the absence of a distance effect for empty sets not only in exclusively selective neurons, but also in the whole population. Moreover, by implementing a decoding approach, we found that VIP neurons rely on low level visual features to identify empty sets. Thus, the parietal encoding of empty sets by VIP neurons lacks the abstract character of numerical representations.

In contrast to VIP, empty set neurons in PFC exhibit a numerical *distance effect* in their tuning. The positioning of empty sets with respect to other numerosities was evidenced by the ordered layout formed by the sample-driven neuronal trajectories in the state space analysis. Particularly, the presence of a significant distance effect for empty sets during the sample period showed that these stimuli were encoded in a quantitative way by PFC. Complementing this finding, a *size effect* emerged in the discriminability of different samples by the classifier in PFC. Moreover, decoding results showed that while the representation of empty sets in prefrontal cortex is invariant to stimulus features. Namely, the SVM classifier in PFC fully generalized across the different visual presentation formats for empty sets. These results point to a quantitative and abstract representation of empty sets in the prefrontal cortex. Then, only the prefrontal encoding of empty sets meets the criteria of a primitive correlate of numerosity zero.

The analysis of error trials offered additional evidence of the differential integration of empty sets as quantitative stimuli in VIP and PFC. While the activity of empty-set neurons in both cortices was relevant for behavioral performance, we found differences in the role played by standard number neurons (preferring countable numerosities) in empty sets trials. Particularly, only the activity of prefrontal number neurons during empty-set presentation affected trial outcomes. The tuning curves of neurons preferring countable numerosities predict a low firing rate for empty sets. However, this low level of activity in response to empty sets is functionally relevant in prefrontal, but not in parietal cortex. This finding further supports the statement that prefrontal neurons integrate empty sets in the stimuli range they encode, together with other numerosities.

6. Are empty sets processed as other numerosity stimuli in the parieto-frontal magnitude system?

Behavioral studies in human and non-human primates suggest that zero is processed differently than countable numerosities. For example, even when pre-scholar children can treat empty sets according to their null value, this is rather an effortful accomplishment. In an ordering task, pre-scholar children still exhibit worse accuracy in trials including empty sets than in standard trials (Merritt and Brannon 2013). Behavioral studies in adults also offer contradictory results. On the one hand, the SNARC effect suggests that zero is represented as a small number, in the left extreme of the mental number line (Stanislas Dehaene, Bossini, and Giraux 1993). On the other hand, reading times of the numeral zero are longer than predicted by its null-numerical value (Brysbaert 1995). Analogously, primate studies consistently report a *distance effect* for empty sets in performance accuracy, but reaction times are either inconsistent or longer than expected (Merritt, Rugani, and Brannon 2009).

6.1 Performance & reaction times

We have previously shown that, even in a delay-match-to-sample numerosity task, Rhesus monkeys treat empty sets as endowed with a zero value. The presence of *distance* and *size effects* for empty sets in the monkeys' performance supported this conclusion. However, empty sets elicited long reaction times, even after extensive training. Particularly, reaction times in empty set trials were longer than in numerosity-one trials. This finding suggests that empty sets are processed differently with respect to other numerosity stimuli. Then, it is possible that the quantitative treatment of empty sets is the result of a different and longer processing in the parieto-frontal magnitude system. To investigate this possibility, we analyzed the temporal dynamics elicited by different stimuli in VIP and PFC.

6.2 The dynamics of number, shape and protocol in VIP and PFC

In order to quantify the strength with which different stimulus factors are encoded by neuronal firing rates in time, we calculated the percentage of explained variance, specifically omega squared (PEV ω^2), from a sliding three-way-ANOVA (200 ms window, 20 ms step, 25 permutations) in both cortical areas.

Figure 6.1 shows the dynamics of ω^2 for factors number, shape and protocol in the whole population of parietal and prefrontal neurons. Information about shape increases sharply when the background (either a circle or a square) is first shown, in the beginning of the fixation period. In contrast, numerosity is available to the monkey only after the sample stimulus is displayed. Then, number information increases more steeply in PFC than in VIP. During the first part of the delay, when the stimulus has disappeared, ω^2 slowly decreases in both areas. Notice that the influence of main factor protocol is more prominent in VIP than in PFC during the sample period, which could be explained by the sensitivity to low level features attributed to parietal cortex.

6.3 Two surges of numerosity selectivity in VIP

Then, we analyzed how numerical selectivity and tuning changed in the course of a trial. For that purpose, we identified the start and end of numerosity selective responses in each neuron taking ω^2 value as parameter. For each factor and their interactions, significance in a time bin was determined by a permutation test for ω^2 (1000 shuffled values, $p < 0.01$). A '*numerosity selective response*' was defined as at least 3 consecutive bins (encompassing a minimum of 240 ms) which were significant for main factor number. The stringency of our criteria was confirmed by the low incidence of numerosity selective responses, so defined, during fixation in both prefrontal and parietal neurons (<1% of responses in the sample period, with the same duration). We identified 320 numerosity selective responses from 228 neurons in the parietal cortex and 424 responses from 276 neurons in the prefrontal cortex. Responses are classified as sample and delay responses according to their timing. Neurons with at least one numerosity selective response are henceforth referred to as '*selective neurons*'. Examples of selective neurons in PFC and VIP are shown in **Figure 6.2**. The red bar at the bottom of the PSTH marks the duration of a numerosity selective response, as previously defined. Response tuning curves are shown in insets. The bottom panel depicts ω^2 for main factors number, shape and protocol along time.

Figure 6.3 A shows ω^2 for numerosity in the population of sample selective neurons in VIP and PFC. Neurons which prefer empty sets (solid lines) are distinguished from those preferring other stimuli (dotted lines). **Figure 6.3A** suggests that, after sample presentation, number selectivity increases faster in prefrontal neurons than in parietal neurons. This finding was confirmed by calculating ω^2 with a high temporal resolution (50 ms kernel, 1 ms shift). In each neuron the selectivity latency was defined by the first of 25 continuous bins with a significant ω^2 for factor number, according to a permutation test (1000 shuffled permutations, $p < 0.05$). We compared the selectivity latencies of neurons that satisfied this criterion during the sample period in the two cortices (98 VIP and 185 PFC neurons).

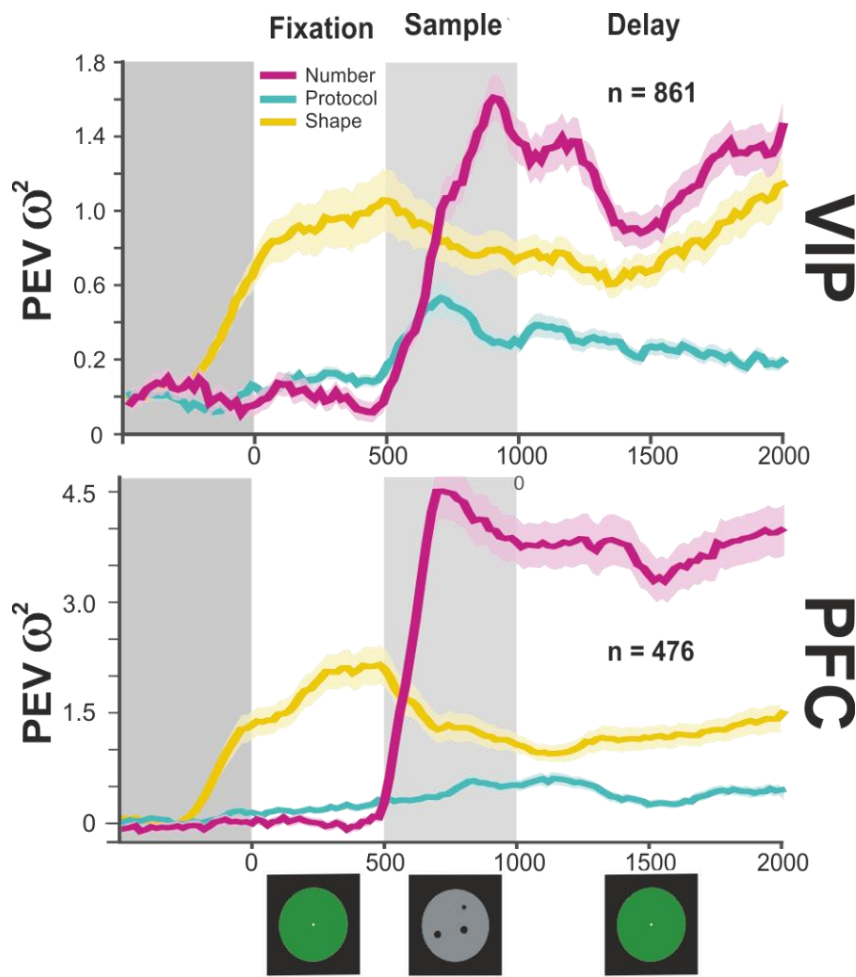


Figure 6.1 PEV (ω^2) in the whole population of VIP and PFC neurons.

Percentage of intertrial variance in firing rates explained by the different stimulus factors (number, protocol and shape).

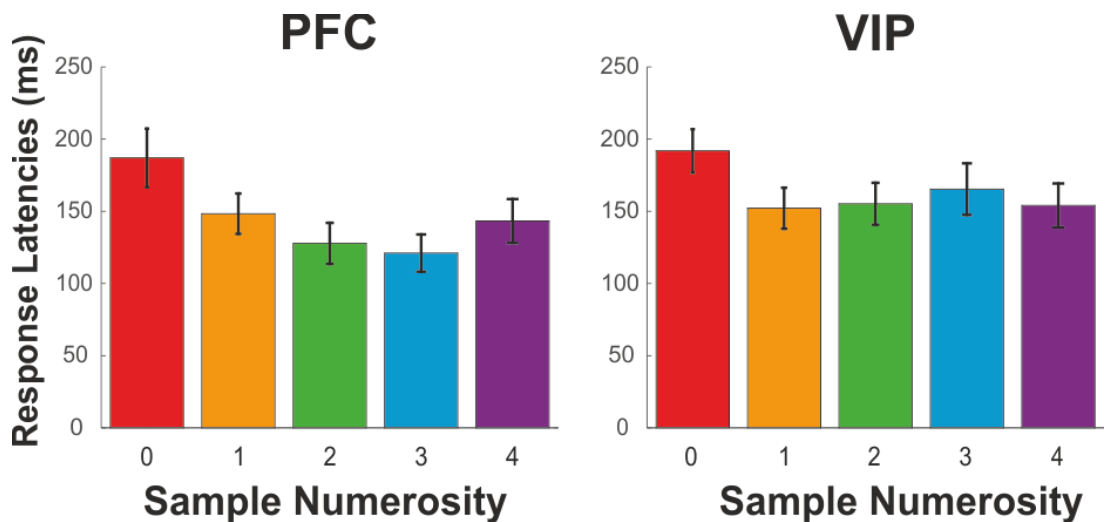


Figure 6.5 Mean response latencies. Mean response latencies for the different sample stimuli in numerosity selective neurons.

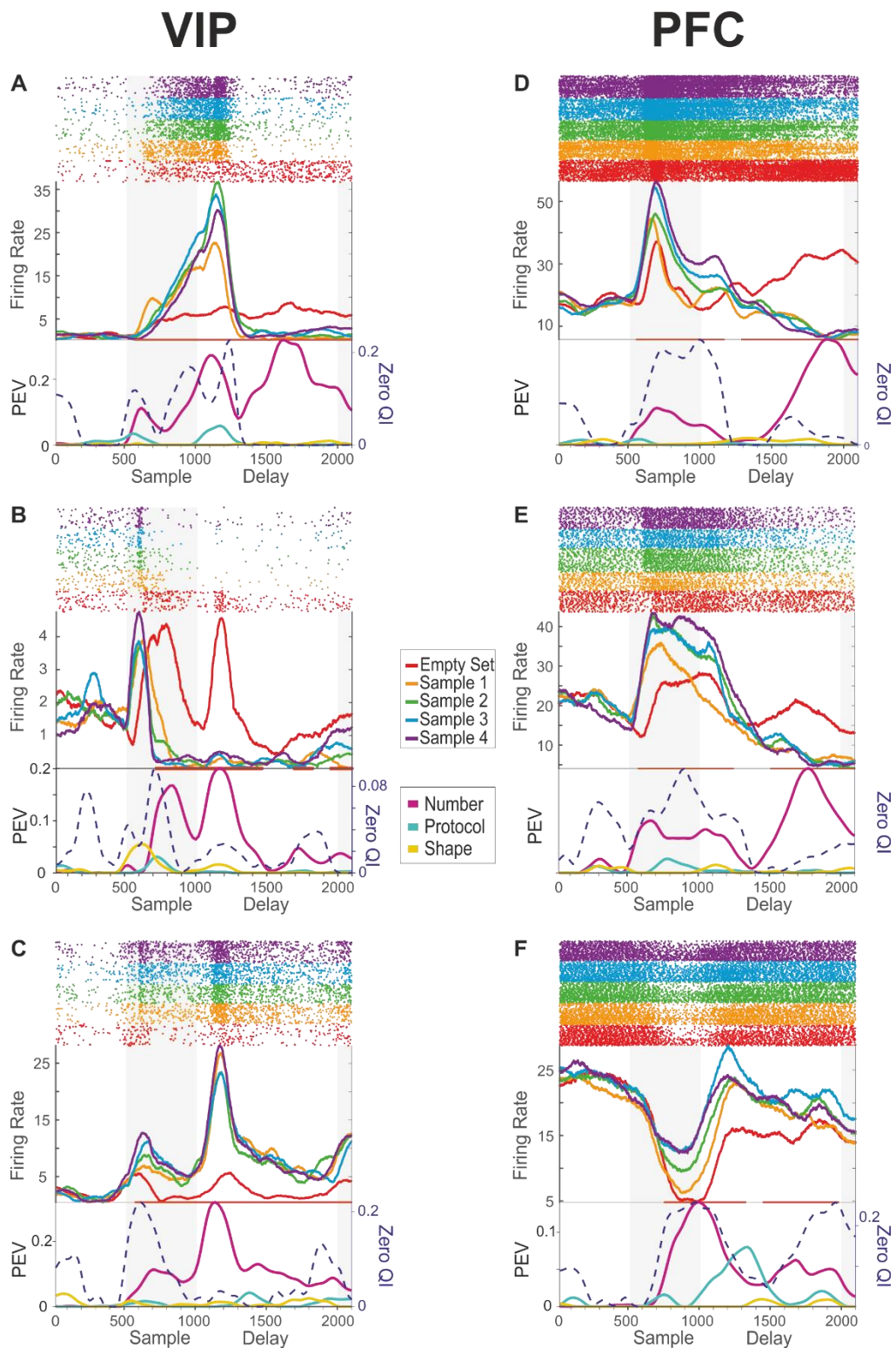


Figure 6.2 Numerosity selective neurons in VIP and PFC. (A-C) Example numerosity selective neurons in VIP. Top panel shows dot-raster histograms (each dot represents an action potential); middle panel depicts averaged spike density functions (activity averaged in a sliding 150 ms window); bottom panel depicts PEV (left axis) for the 3 main factors number (pink), protocol (blue) and shape (yellow); and the index QI (right axis, dotted line). The first 500 ms represent the fixation period, followed by the sample and delay periods. Inset in spike density plot shows the neuron's tuning function (i.e., discharge rates as a function of the number of presented items) during the grey shaded sample period. (D-F) Example numerosity selective neurons in PFC.

Sample selective neurons

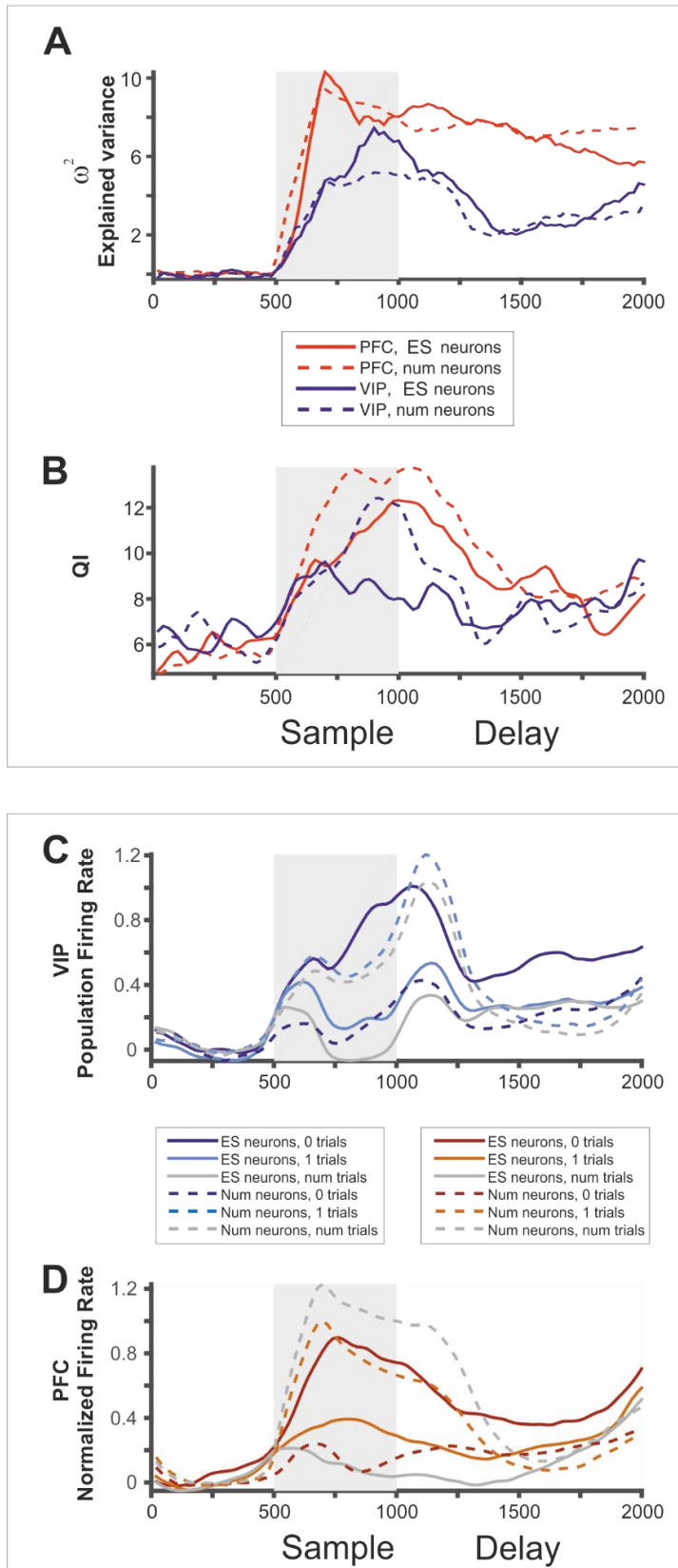


Figure 6.3 Selectivity, tuning and response dynamics in numerosity selective neurons.

(A) Dynamics of numerosity information in sample selective neurons. (B) Temporal dynamics of QI in sample selective neurons (C-D) Average firing responses for different types of stimuli (Empty sets, numerosity 1 and larger numerosities) in empty set preferring neurons (solid lines) and countable numerosity preferring neurons (dotted lines) in VIP (C) and PFC (D).

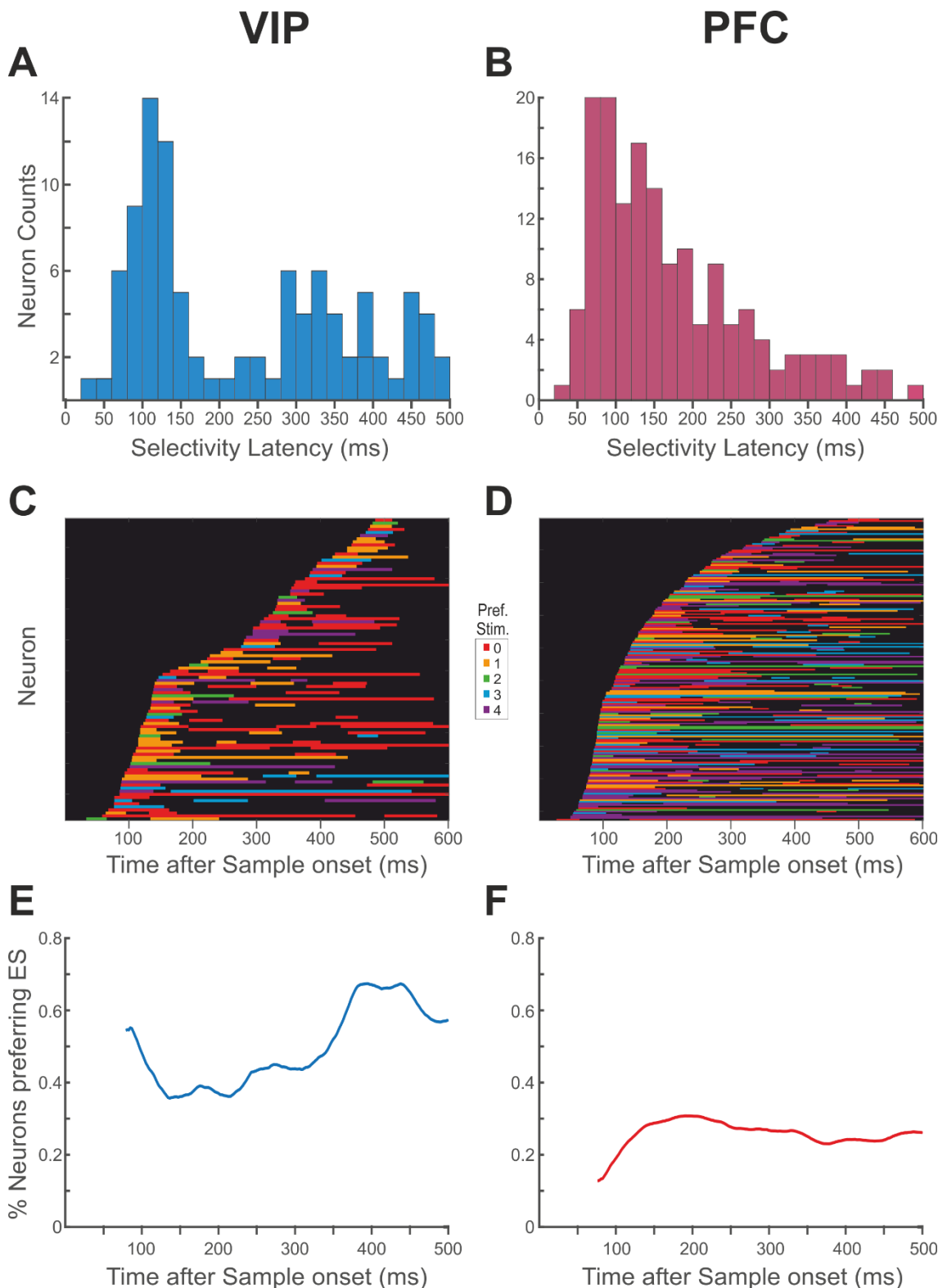


Figure 6.4 Selectivity latencies and numerosity preference during the sample period. (A&B) Distributions of number selectivity latencies in VIP and PFC neuronal populations. The distribution of parietal selectivity latencies in VIP is significantly bimodal. **(C&D)** Neurons are ordered according to their selectivity latency and selective responses during the sample period are colored according to stimulus preference. **(E&F)** Percent of selective responses having empty sets as preferred stimulus along the sample period.

Surprisingly, in average, parietal neurons showed longer selectivity latencies than prefrontal neurons (230.6±13.7 ms in VIP versus 160.7±7.3 ms in PFC, $p < 0.001$, Mann-Whitney U-test).

In previous studies, which did not include empty sets, we had consistently found the opposite: shorter latencies in parietal cortex than in prefrontal cortex. However, in the present study, parietal selectivity latencies exhibit a significantly bimodal distribution (**Figure 6.4A**, Hartigan dip test, $p < 0.05$). There are two surges of parietal neurons becoming selective, by first time, during the sample period. In contrast, selectivity latencies in PFC are unimodally distributed (**Figure 6.4B**). Similar results were obtained by defining selectivity latencies differently or using alternative methods (AUROC between activity for the most and least preferred numerosities).

Figure 6.4C&D show the cumulative distributions of selectivity latencies in VIP and PFC, respectively. Selective responses, defined in this high definition ω^2 analysis, are color coded according to their preferred stimulus. **Figure 6.4E&F** show the percent of selective responses in the population which prefer empty sets along the sample period. In VIP, soon after sample presentation, this proportion starts increasing in a steady way (**Figure 6.4E**). In contrast, the percent of responses preferring empty sets slowly decreases in PFC (**Figure 6.4F**) during the sample period.

6.4 Late neuronal responses for empty sets

Selectivity latencies reflect the time at which neurons start discriminating between different stimuli. To explore the neuronal dynamics particularly elicited by empty sets we calculated visual latencies in neurons with numerosity selective responses in the sample period, defined by (133 VIP neurons and 205 PFC neurons). Examination of single-cell neurons in VIP and PFC suggested that, in comparison to other stimuli, empty sets evoke late neuronal responses (**Figure 6.2 B,D,E**). To test this possibility, in each neuron, we sorted trials according to the type of sample stimulus and calculated response latencies. **Figure 6.5** shows (page 51) the average response latencies for the different numerosities in the population of VIP and PFC neurons.

6.5 Empty sets elicit a distinct temporal response profile

Then, we explored the response dynamics elicited by different stimuli in VIP and PFC (**Figures 6.3 A-D**). For that purpose, firing rates were calculated in a sliding 200 ms window, which was stepped in 20 ms intervals over the course of the trial. Trial firing rates were z-scored, in each neuron, by subtracting the mean baseline rate

and dividing by the baseline standard deviation across trials plus a correction factor (0.1). Then, average firing rates were calculated for the different sample stimulus (empty sets, numerosity one and other numerosities). Neurons which prefer empty sets (solid lines) were distinguished from those preferring other stimuli (dotted lines).

Approximately, two hundred milliseconds after stimulus presentation, the response of prefrontal empty set neurons reaches a peak and slowly drops off afterwards (**Figures 6.3 D**). A very different view emerges in VIP. The temporal response profile of parietal empty set neurons differs in trials involving empty sets and other stimuli (**Figures 6.3 C**). When a set containing dots is presented as sample, empty-set preferring neurons in VIP show an early phasic response with firing rates slowly declining in the late sample period. In empty set trials, only the first part of this response is preserved. Approximately 200 ms after an empty set is presented the population firing rate stops its decline and starts rising. This sudden change in response dynamics during the sample period can also be observed at the single neuron level (**Figures 6.6**). Some parietal neurons start responding early after sample presentation and suddenly change their tuning later (i.e. **Figure 6.6 A-D, F**), while other neurons respond by first time later in the epoch (i.e. **Figure 6.6 E**). This latter group explains the bimodal distribution in selectivity latencies in VIP.

6.6 A dynamic shift in the population tuning towards a categorical representation of empty sets

The differences in responses elicited by empty sets and other stimuli suggest that population tuning is changing in both cortical areas along the course of a trial. To quantify how numerosity tuning changed in VIP and PFC during the course of a whole trial, we derived time-defined tuning curves from sliding time windows (200 ms duration, 20 ms step) in each neuron. Then, we performed tuning-curve cross-correlation (Diester & Nieder 2008) between subsequent pairs of tuning curves. This method quantifies the extent to which tuning curves change from one bin to another. **Figures 6.7 A&B** show CC values in the course of a trial in VIP and PFC populations of sample selective neurons. A decrease in CCs values represent a change in neuronal tuning. **Figures 6.7 C&D** show the mean CCs along time in the populations of VIP and PFC sample selective neurons.

Interestingly, we found that 200 ms after sample presentation, tuning curves change importantly in many VIP sample selective neurons. This is reflected in a negative deflection in **Figure 6.7 C**. This finding suggests that at this moment of the trial the tuning of selective parietal neurons suddenly changes in VIP.

VIP neurons

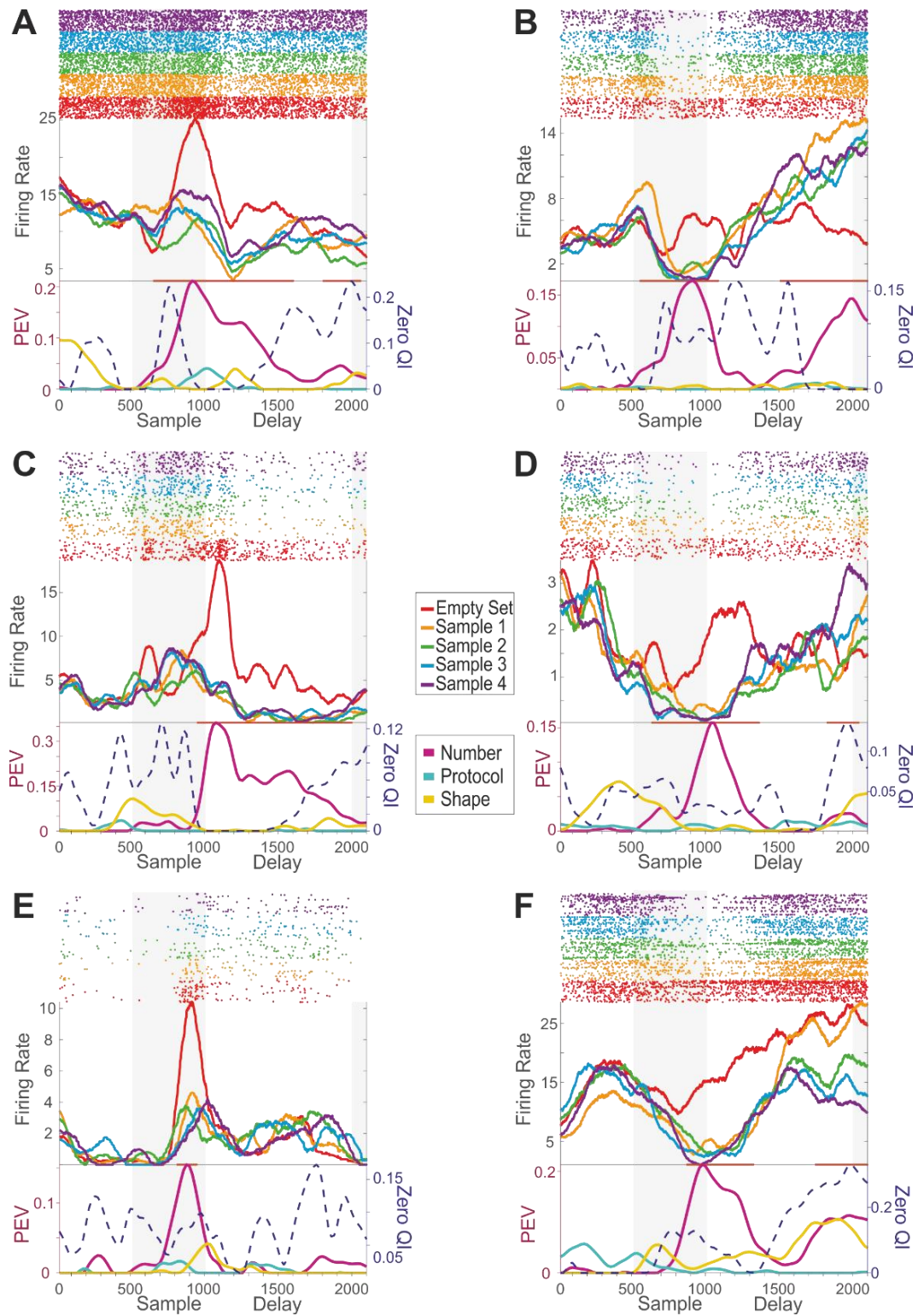


Figure 6.6 Empty sets elicit a change in parietal single-neuron response dynamics during the sample period. In empty set trials the response of numerosity selective neurons in VIP changes during the sample period.

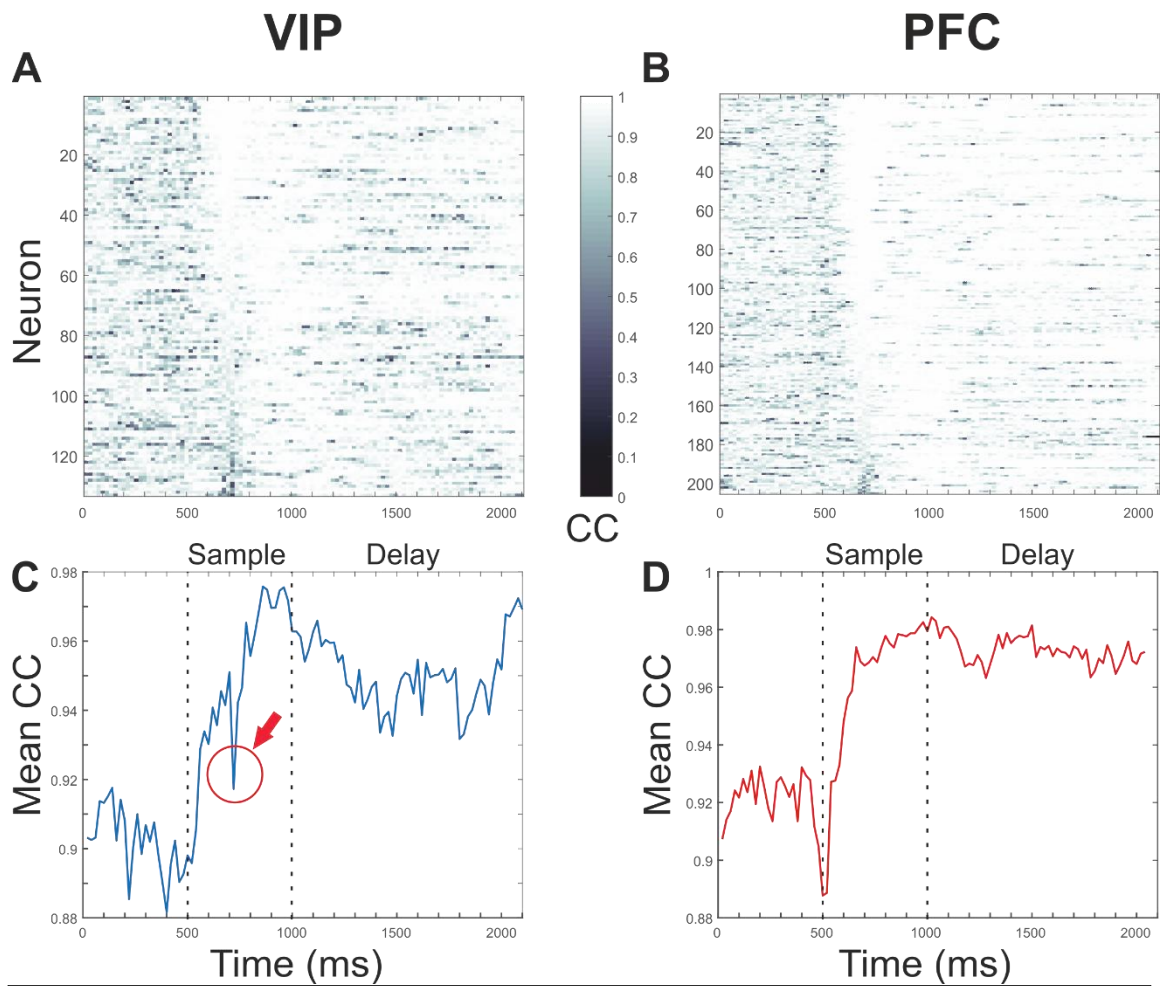


Figure 6.7 Tuning Curve Cross-correlations along time. (A&B) Cross-correlation values between subsequent tuning curves in VIP and PFC individual neurons. **(D&E)** Average cross-correlation values in the population on VIP and PFC neurons.

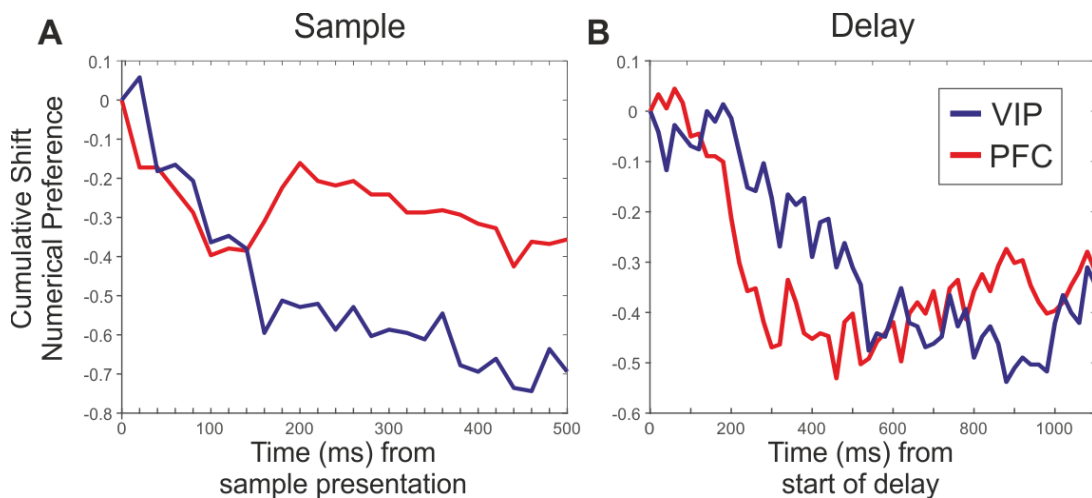


Figure 6.8 Mean cumulative shift in numerical preference. (A) Average cumulative change in numerical preference during the sample period in the populations of sample selective neurons in VIP and PFC. **(B)** Mean cumulative change during the delay epoch in the populations of delay selective neurons in VIP and PFC.

Then, we investigated in which respects the tuning curves evolved in time. Particularly, tuning variations can be described in terms of a change in preferred numerosity and tuning profile (categorical versus quantitative). At the population level, stimulus preference shifts towards empty sets in the course of a trial (**Figure 6.8**). Particularly, **Figure 6.8A** shows the change in numerical preference during stimulus presentation in VIP and PFC sample selective neurons. The accumulated shift towards empty sets is more prominent in VIP than in PFC. **Figure 6.8B** depicts the cumulative shift in both cortical areas during the subsequent 1-second period, when the stimulus is not visually available anymore, in delay selective neurons.

Notice that, independently of their numerosity preference, a neuron can have either a binary or a graded tuning profile for empty sets. We used our quantitative index (QI) to explore this aspect of tuning. High values reflect a more graded tuning, while low values correspond to a more binary tuning (nothing versus something) for empty sets. **Figure 6.3B** depicts how this index evolves in time in both cortical areas. After sample presentation, QI initially increases in VIP and PFC empty set neurons. However, approximately 200 ms later, QI suddenly starts decreasing in this population (**Figure 6.3B**, blue solid line). This pattern suggests that, in the middle of the sample period, parietal tuning becomes more categorical. In contrast, QI continues rising in prefrontal neurons in the late sample period. In the delay period tuning becomes gradually more categorical with respect to empty sets in both cortical areas. Crucially, this is not the case for other individual numerosities.

6.8 Differential contributions of PFC and VIP to behavior

We furthermore explored whether the strength of numerosity representation in selective neurons, irrespective of their stimulus preference, is related with successful completion of the task. To that end, we compared the amount of information about numerosity carried by neurons when monkeys made mistakes and when they responded correctly. As measure, we quantified ω^2 explained variance in number selective neurons with a sufficient number of error trials per stimulus numerosity, including empty sets (VIP 12 neurons, PFC 19 neurons, **Figure 6.9A&B**). Omega squared was compared between correct and error trials, in both areas, after averaging across neurons in specific trial periods (sample and delay). If the encoding of numerosity in exclusive selective neurons is relevant for behavior, the amount of information they carry could predict the execution of mistakes by the monkeys. We found that, in error trials, ω^2 is reduced in prefrontal neurons during both the sample and delay periods (Sample: 4.14 ± 1.13 versus 1.07 ± 0.5 , $p=0.005$; Delay: 5.26 ± 1.12 versus 1.93 ± 0.58 , $p=0.039$, for correct and error trials, respectively, Wilcoxon signed-rank test). In parietal neurons, we did not find outcome-related differences in ω^2 (Sample: 3.86 ± 1.33 versus 5.63 ± 2.53 , $p=0.922$; Delay: 3.23 ± 0.09 versus 4.43 ± 2.66 ,

$p=0.38$, for correct and error trials) (**Figure 6.9**). This finding suggests behavior is predominantly influenced by the strength of numerosity representation in PFC.

6.9 Characterization of empty set preferring neurons

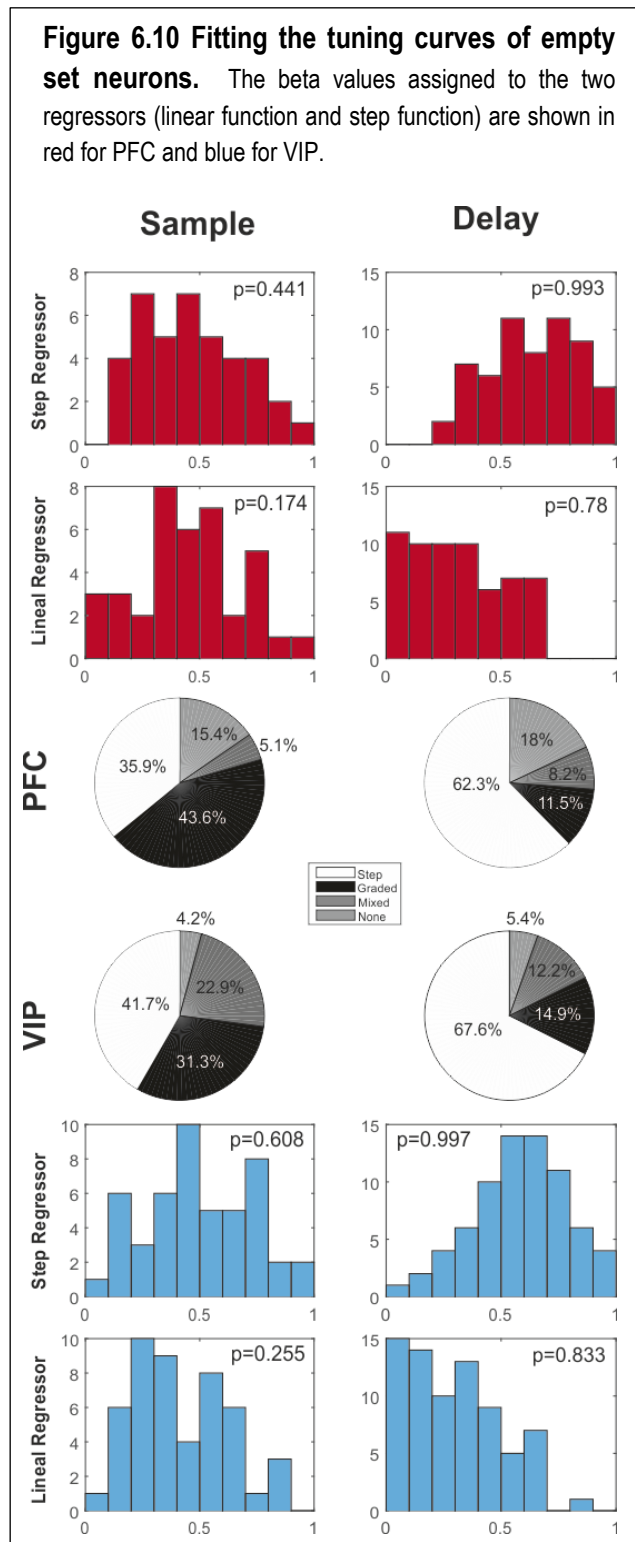
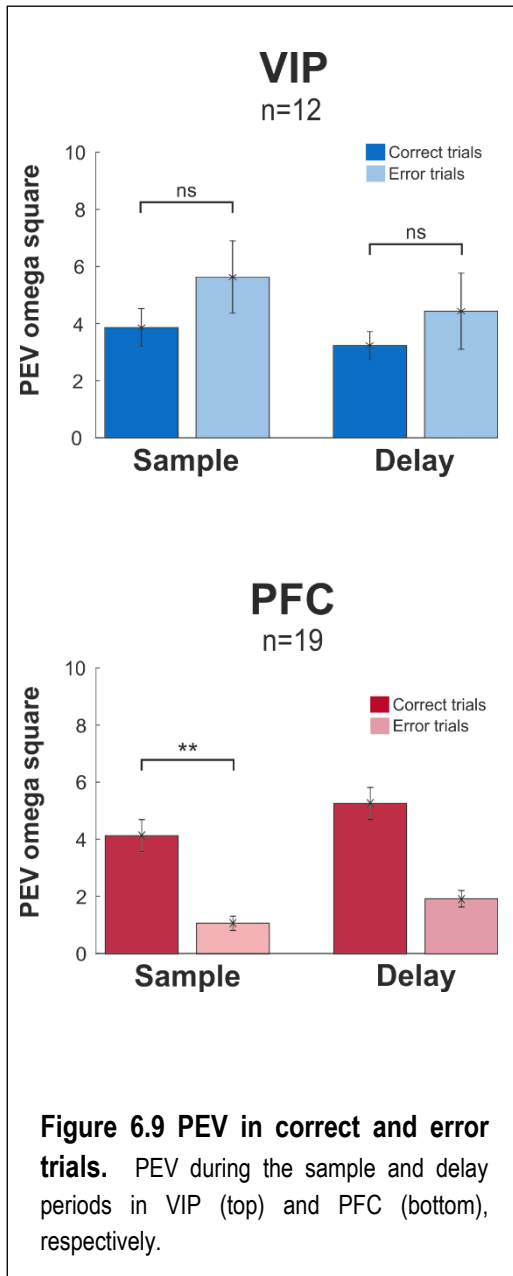
A recent report from one monkey postulated that, according to their tuning, two distinct types of empty set preferring neurons can be identified in the parietal cortex (Okuyama, Kuki, and Mushiake 2015). The authors characterize such types of neurons with respect to their tuning properties, which correspond to what we have called ‘binary-categorical’ and ‘graded-quantitative’ tuning.

6.9.1 *A continuum tuning profile of empty-set preferring responses*

In order to approach this question we fitted the tuning curve of each empty-set preferring response with a generalized linear model (GLM) that contained a linear combination of two regressor functions: a linear equation and a step-like function (See **Figure 6.10**). We analyzed the distributions of beta values across empty-set-preferring responses. These values correspond to the weight assigned to each regressor in the GLM and therefore reflect to what extent a particular tuning curve can be described as the corresponding category. A bimodal distribution of beta values would suggest that two types of empty set responses can be identified according to their tuning functions. However, for both regressors, the beta values were unimodally distributed (Hartigan dip test, 1000 bootstrappings). That was also the case when we looked at the distribution of the sigmas fitted in the Gaussian equation. These results already suggest that two distinct types of responses preferring empty sets cannot be clearly differentiated. Thus, we conclude that empty-set preferring responses are situated in the poles of a continuous progression.

Following the approach used by (Engel et al. 2015), we classified the tuning profile of responses. In comparison to VIP, PFC shows a lower percent of empty set neurons with a continuous tuning profile and a higher percent of neurons with a categorical profile in the sample period. However, these differences did not reach statistical significance (Step 35.9% versus 41.7%, $X^2(1,87)=0.3$, $p=0.583$; Lineal 43.6% versus 31.3%, $X^2(1,87)=1.4$, $p=0.235$, in PFC and VIP, respectively).

Note that, from sample to delay, beta values assigned to the step-function increase while those assigned to the lineal function decrease significantly in both cortical areas (PFC: step function 0.472 ± 0.035 vs. 0.648 ± 0.028 , $p=0.00048$; lineal



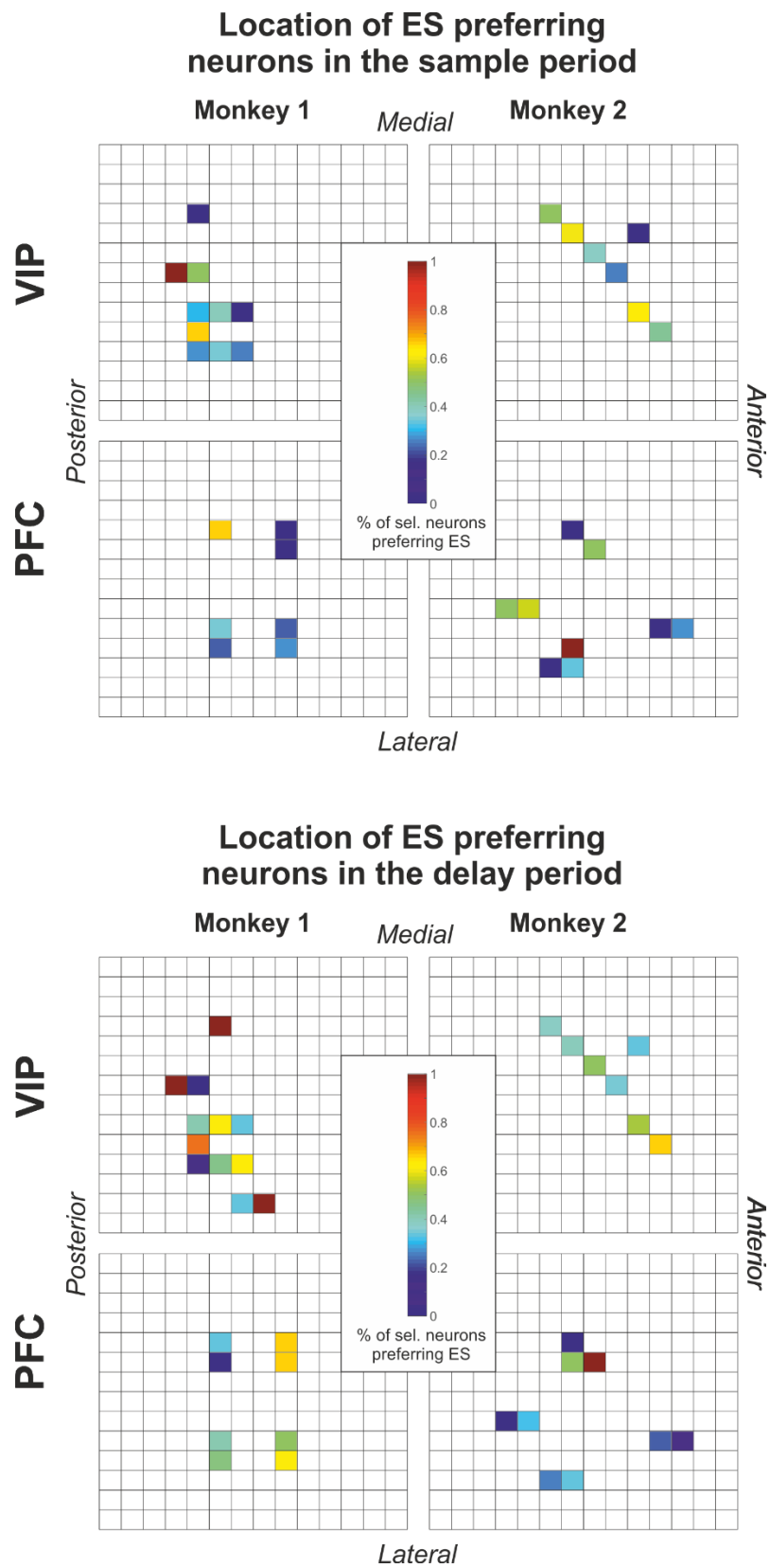


Figure 6.11 Location of numerosity selective neurons preferring empty sets in VIP and PFC. The proportion of selective neurons responding maximally to empty sets during the sample (top) or delay period (bottom) is shown color-coded for the different recording locations.

function 0.475 ± 0.04 vs. 0.318 ± 0.025 , $p=0.0031$. VIP: step function 0.507 ± 0.035 vs. 0.596 ± 0.024 , $p=0.041$; lineal function 0.417 ± 0.03 vs. 0.298 ± 0.024 , $p=0.0023$, for sample and delay, respectively, Mann-Whitney U-test). This pattern confirm that empty sets are represented in a more categorical fashion during the delay period.

6.9.2 Location of empty set preferring neurons

Neuroimaging studies (Harvey et al. 2013) have described a topographic representation of numerosity in the parietal cortex. We found no evidence that neurons with empty-set preferring responses were clustered in a certain location of VIP or PFC (**Figure 6.10**). This holds true when selectivity and numerical preference are derived from activity in different trial epochs (whole sample or delay) or by defining selective responses in time. However, it is worth noting that our recordings only allow us to sample small extensions of the parietal or prefrontal cortex with a few channels (max. 8 channels per area in one session). Therefore, our negative results should be interpreted cautiously.

7. Discussion

We investigated the neuronal representation of empty sets in two monkeys trained to perform a delayed match-to-sample task using empty sets and countable numerosities as stimuli. Behavioral performance confirmed that monkeys assess empty sets according to the null quantity they convey. Simultaneous recordings from VIP and PFC showed that, as for countable numerosities (1-4), a high proportion of selective neurons are tuned to empty sets. Importantly, prefrontal population responses exhibited both a distance and a size effect for empty sets. In contrast, parietal population responses to empty sets lacked such quantitative traits and distinguished empty sets from all other stimuli classes in a categorical fashion. Moreover, decoding results showed that while the representation of empty sets in prefrontal cortex is invariant to stimulus features, their parietal representation is driven to a larger extent by visual properties. Therefore, only the prefrontal encoding of empty sets meets the criteria of a primitive correlate of numerosity zero.

7.1 Monkeys treat empty sets as conveying a null quantitative value

The performance of both monkeys suggested that empty sets were positioned closer to numerosity 1 than to numerosity 2 on the monkeys' mental number line. This finding, a behavioral distance effect, signals a representational continuity between empty sets and countable numerosities. Our behavioral results are in agreement with the conclusions of behavioral studies in chimpanzees (Beran 2012) and monkeys (Merritt, Rugani, and Brannon 2009). Particularly, our data corroborates the findings of a study in which the accuracy of rhesus monkeys in matching and ordering tasks revealed a distance effect for empty sets (Merritt, Rugani, and Brannon 2009). The effect appeared in non-differentially reinforced trials and, importantly, prevailed when extensive controls were imposed (background size, background color, items color). Our data extends such results by showing that a behavioral distance effect for empty sets also emerges when the test stimulus is not simultaneously, but sequentially presented. More importantly, our results show that this effect does not completely extinguishes with extensive training (differentially rewarded trials).

Interestingly, behavioral findings in monkeys are reminiscent of the way pre-school children treat empty sets (Merritt and Brannon 2013). Moreover, a distance effect for empty sets has also been shown in adult numerate humans (Merritt and Brannon 2013).

Humans and non-human primates treating empty sets similarly points to a common primitive and non-symbolic representation of null-quantity. Such pre-symbolic representation might constitute a precursor of the human concept of 'zero'.

7.2 Differential tuning for empty sets in VIP and PFC

To test how the brain represents empty sets, we simultaneously recorded single neuron activity from VIP and PFC in two behaving monkeys. These cortical regions have been shown to play cardinal roles in numerosity discrimination in animals and humans (Harvey et al. 2013; Piazza et al. 2004; Nieder and Dehaene 2009). As reported in previous studies (Nieder and Miller 2004; Viswanathan and Nieder 2013; Nieder and Miller 2003), we found neurons tuned to countable numerosities in both cortical areas. However, we also identified a relatively high proportion of neurons that responded maximally to empty sets. Importantly, to exclude the effect of appearance (i.e. shape) and low level visual properties (i.e. dot density, total dot area, total stimulus luminance) on numerosity tuning, only 'exclusive' number neurons were considered to investigate the representation of empty sets at the single neuron level.

A differential tuning for empty sets emerged between the two cortical areas. Empty-set neurons in VIP barely discriminated countable numerosities, failing to exhibit a neuronal distance effect. The absence of a progressive drop-off of activity with numerical distance disqualifies the characterization of these neurons as numerosity tuned. The resulting binary-like tuning profile can be better described as signaling the presence or absence of countable items.

Recently, Okuyama et al. (Okuyama, Kuki, and Mushiake 2015) reported VIP neurons which were tuned to the absence of countable stimuli in one monkey. This monkey was trained to assess the numerosity of a target display (that could show no items) and add or subtract items in a second display to match the target numerosity. These authors classified neurons which responded maximally to empty sets into two distinct groups: 'exclusive-discrete types' which showed no modulation to numerosities 1 to 4 (based on an ANOVA), and 'continuous types' that exhibited a significant response to numerosity 1. Two-thirds of the empty-set neurons they recorded in VIP were classified as a discrete type and the rest as a continuous type. No further statistical tests were applied to explore whether these cells belonged to two distinct classes. Even when the exclusive-discrete type of tuning was predominant in the population, the authors asserted that parietal neurons represent numerosity zero. Furthermore, of the representation to image and luminance related parameters was not addressed.

In our recordings from two monkeys, we found an even higher proportion of 93% VIP selective empty set neurons that belonged to the 'discrete type' class according to the definition of Okuyama et al. (Okuyama, Kuki, and Mushiake 2015). Additionally, we found no evidence for two strict classes of empty-set neurons in VIP but rather a continuum of more discrete to more continuous empty-set detectors. In both studies the neuronal recordings were derived from an initial 'target phase', when

the numerosity the monkey had to match at the end of the trial was displayed. Task demands seem comparable at this initial point of the trial, and therefore could hardly account for results discrepancies. Rather, differences in stimulus protocols and data processing might account for the observed discrepancies. Particularly, we excluded the effect of visual stimulus features (luminance level and shape) on neuronal responses to numerosity. Consequently, we found that VIP neurons represent empty sets primarily as a separate category, distinct from all countable numerosities.

Our population analysis confirmed that VIP does not genuinely represent empty sets as part of the numerosity continuum. First, analyses at the population level showed that a binary tuning is present in VIP neurons, irrespective of their selectivity and stimulus preference. Specifically, the population state space analysis (GPFA) confirmed the absence of a distance effect for empty sets not only in exclusively selective neurons, but also in the whole population. Second, by implementing a decoding approach, we found that VIP neurons rely on low level visual features to identify empty sets. Thus, the parietal encoding of empty sets by VIP neurons lacks the abstract character of numerical representations.

The discrepancy between behavior and parietal population tuning could be resolved by the responses of prefrontal neurons. In contrast to VIP, empty-set neurons in PFC showed a gradual drop-off of activity with increasing numerosity. Moreover, at the whole population level, the positioning of empty sets with respect to other numerosities was evidenced by a significant distance effect in the state space analysis. The ordered layout formed by the sample-driven neuronal trajectories during the sample period showed that empty sets were encoded in a quantitative way by PFC. Complementing these findings, a 'size effect' emerged in the discriminability of different samples by the classifier in PFC. These results suggest that the prefrontal cortex does integrate numerosity zero as the lower end of the numerical continuum.

7.3 Behavioral relevance of parietal and prefrontal representations of empty sets

The analysis of error trials also points to a differential integration of empty sets as quantitative stimuli in VIP and PFC. While the activity of empty-set neurons in both cortices was behaviorally relevant, we found differences in the role played by standard number neurons (preferring countable numerosities) in empty sets trials. Particularly, only the activity of prefrontal countable-numerosity neurons during empty-set presentation affected trial outcomes. The tuning curves of neurons preferring countable numerosities predict a low firing rate for empty sets. However, the reduced response of these neurons to empty sets is functionally relevant in prefrontal, but not in parietal cortex. This finding further supports the statement that prefrontal neurons integrate empty sets in the stimuli range they encode, together with other numerosities.

In addition, as for behavioral performance functions, a logarithmic number line accounted better for the tuning curves of numerosity selective neurons in PFC, but not in VIP. Thus, neuronal activity in the prefrontal cortex shows a stronger correlation with behavior than parietal activity.

7.4 Level of invariance of empty-set representations to stimulus features

Some degree of abstraction is required from any neural correlate of numerosity. Therefore, we would expect a neural representation of empty sets to be invariant to image-like and low level features of the stimuli. In our task, we varied background shape and controlled for luminance. Neurons sensitive to these non-numerical parameters were excluded from single-neuron analyses. Moreover, at the population level, a decoding approach allowed us to explore the invariance of empty set representations in VIP and PFC. We found that the discrimination of empty sets by parietal neurons was reduced across stimulus features. This finding could be explained by a mixture of visual and numerical selectivity in VIP. In contrast, PFC represented empty sets abstractly, as evidenced by high decoding performance across stimulus properties.

Previously, the idea of abstract number representations was deemed premature based on some behavioral and human functional imaging studies (Cohen Kadosh and Walsh 2009). Over the past years, however, neurons indiscriminant to spatio-temporal and cross-modal number variations have been found, particularly in the PFC (Nieder, Diester, and Tudusciuc 2006; Nieder 2012). Recent human imaging studies also report that the extraction of numerosity is only minimally influenced by the processing of physical stimulus features (Park et al. 2015). These findings suggest that at least some neurons in association cortices represent numerosities abstractly. Of course, abstract number information could also be extracted from population activity as evidenced by the analyses presented in the current study. Still, whether PFC neurons encode empty-sets in different formats (across modalities and spatio-temporal presentation) requires further investigation.

7.5 Numerosity zero in the labeled line code for number

Several computational models of numerosity detection operate with intermediate-stage summation units that show monotonically increasing or decreasing discharges as a function of number (also found in area LIP (Roitman, Brannon, and Platt 2012)) before giving rise to peak-tuned numerosity detectors at the output stage. Being zero the smallest numerosity tested, empty-set cells show decreasing rate functions reminiscent of decreasing summation units. On average, however, their tuning curves were too selective (i.e., narrow) to render them suitable

graded summation units over a range of numerosities. This suggests that empty-set preferring neurons are better considered as detectors tuned to numerical value 0. Additionally, during the sample period, we found an important number of neurons preferring intermediate numerosities (1 to 3). Those high percentages are higher than expected by noise in a neuronal population which is monotonically tuned to number. Finally, we obtained time-defined numerosity tuning curves and performed spectral clustering analysis on them. Five clusters of tuning curves, rather than two, account better for the diversity of numerosity tuning.

Conversely, the class of frequent neurons tuned to numerosity 4 may mirror increasing summation units. However, this class could include neurons preferring higher numerosities whose tuning curves has not been completely sampled. When broader ranges of numerosities (1 to 30) are tested, numerosity tuning preference becomes evenly distributed (Nieder and Merten 2007), supporting the notion that numerosity selective cells in VIP and PFC are essentially tuned to specific numerical values.

7.6 Numerosity zero in a logarithmic numerical scaling

We have previously reported that behavioral and neuronal representations of numerosity in monkeys and crows (Ditz and Nieder 2015; Ditz and Nieder 2016) are best described on a non-linearly compressed, logarithmic number scale. This finding is confirmed in the current study with a new set of data. The logarithmic scheme accounts for the decrease in the discrimination of two stimuli when their magnitude increases (as predicted by Weber-Fechner psychophysical law). A non-linearly compressed scaling of numerosity has the advantage of providing scale-invariance and preference-independent neuronal variability. Even though the logarithm of 0 is not defined, the differences between numerical values can still be represented on a log scale. Note that Weber-Fechner law is concerned with the perception of differences, rather than absolute magnitudes. Starting with the interval between numerosity 0 (n) and numerosity 1 ($n+1$), all differences between higher numbers can be represented on a log scale. Representations of cardinality 0 would therefore not dispute the notion of a nonlinearly compressed scaling.

7.7 From 'nothing' to 'zero'

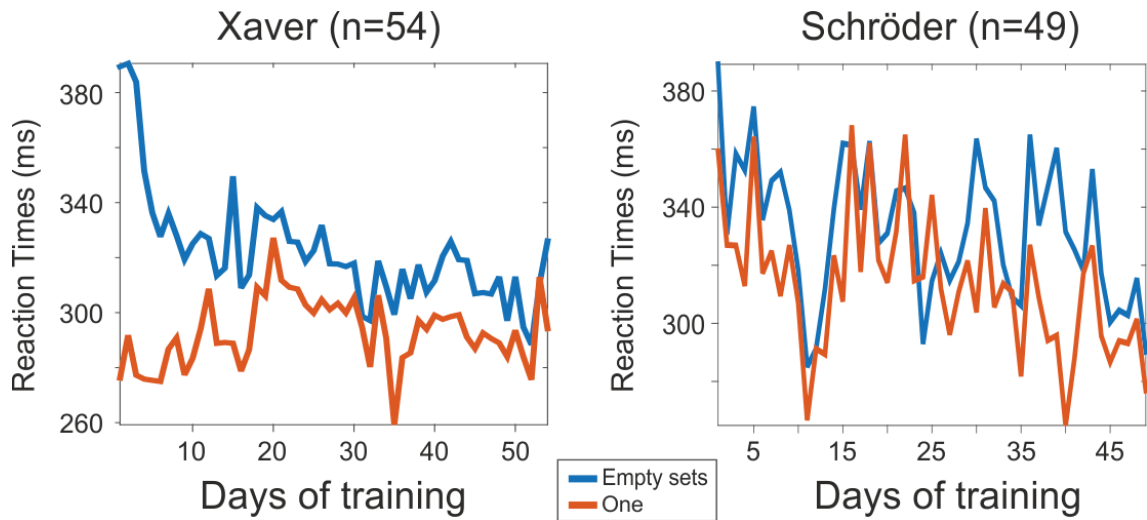
Sense organs have evolved to encode the intensity of a stimulus. Then, how can the absence of stimulation be detected? In order to make use of this information, the nervous system needs to encode it actively. Indeed, it has been shown that neurons in the frontal lobe increase their discharge rate to the categorical absence of a stimulus (Merten and Nieder 2012; Merten and Nieder 2013). Zero is an example of information conveyed by the lack of a signal. In this case, the brain generates a quantitative representation ('zero') from the absence of a behaviorally relevant

sensory signal ('nothing'). This process that would require a high level of cognitive control. In this context, it may not be surprising that neurons in the PFC are particularly engaged in the representation of null-quantity.

It has been argued that the conceptual demands imposed by representing 'nothing' as a numerical category may explain the delayed discovery of zero in human history. Zero first appeared as a placeholder symbol in notational systems. Only later, Indians used zero also as a numeral signifying null quantity in mathematics. This cultural delay is mirrored in ontogeny: children seem to master the cardinal and ordinal properties of small numbers before they can deal with zero (Wellman and Miller 1986). Still, it has been suggested that pre-scholar children understand the numerical value of numerosity zero and position empty sets in the context of other small numerosities before they have developed a concept of symbolic zero (Merritt and Brannon 2013). These results suggest that the representation of empty sets as non-symbolic carriers of null-quantity can be grasped by children and some animals. Our results suggest that a humble precursor of the non-symbolic zero can be identified in the primate prefrontal cortex.

8. Supplementary Figures

Figure S1



10. References

- Beran, Michael J. 2012. "Quantity Judgments of Auditory and Visual Stimuli by Chimpanzees (Pan Troglodytes)." *Journal of Experimental Psychology: Animal Behavior Processes* 38 (1): 23–29. doi:10.1037/a0024965.
- Bialystok, Ellen, and Judith Codd. 2000. "Representing Quantity beyond Whole Numbers: Some, None, and Part." *Canadian Journal of Experimental Psychology/Revue Canadienne de Psychologie Expérimentale* 54 (2). Canadian Psychological Association: 117–28. doi:10.1037/h0087334.
- Biro, Dora, and Tetsuro Matsuzawa. 2001. "Use of Numerical Symbols by the Chimpanzee (Pan Troglodytes): Cardinals, Ordinals, and the Introduction of Zero." *Animal Cognition* 4 (3-4): 193–99. doi:10.1007/s100710100086.
- Bongard, Sylvia, and Andreas Nieder. 2010. "Basic Mathematical Rules Are Encoded by Primate Prefrontal Cortex Neurons." *Proceedings of the National Academy of Sciences of the United States of America* 107 (5): 2277–82. doi:10.1073/pnas.0909180107.
- Boysen, S T, and G G Berntson. 1989. "Numerical Competence in a Chimpanzee (Pan Troglodytes)." *Journal of Comparative Psychology (Washington, D.C. : 1983)* 103 (1): 23–31. <http://www.ncbi.nlm.nih.gov/pubmed/2924529>.
- Brannon, E M, and H S Terrace. 1998. "Ordering of the Numerosities 1 to 9 by Monkeys." *Science (New York, N.Y.)* 282 (5389): 746–49.
- Brysbaert, Bart. 1995. "Arabic Number Reading: On the Nature of the Numerical Scale and the Origin of Phonological Recoding." *Journal of Experimental Psychology: General* 124 (4): 434–52.
- Burr, David, and John Ross. 2008. "A Visual Sense of Number." *Current Biology* 18 (6): 425–28. doi:10.1016/j.cub.2008.02.052.
- Cantlon, Jessica F, Elizabeth M Brannon, Elizabeth J Carter, and Kevin A Pelphrey. 2006. "Functional Imaging of Numerical Processing in Adults and 4-Y-Old Children." *PLoS Biology* 4 (5): e125. doi:10.1371/journal.pbio.0040125.
- Castelli, Fulvia, Daniel E Glaser, and Brian Butterworth. 2006. "Discrete and Analogue Quantity Processing in the Parietal Lobe: A Functional MRI Study." *Proceedings of the National Academy of Sciences of the United States of America* 103 (12): 4693–98. doi:10.1073/pnas.0600444103.
- Chang, Chih-Chung, and Chih-Jen Lin. 2011. "LIBSVM: A Library for Support Vector Machines." *ACM Transactions on Intelligent Systems and Technology* 2 (3): 27:1–27:27. Software available at <http://www.csie.ntu.edu.tw/~cjlin/libsvm>.
- Cohen Kadosh, Roi, and Vincent Walsh. 2009. "Numerical Representation in the Parietal Lobes: Abstract or Not Abstract?" *The Behavioral and Brain Sciences* 32 (3-4): 313–28; discussion 328–73. doi:10.1017/S0140525X09990938.
- Dehaene, S. 1996. "The Organization of Brain Activations in Number Comparison: Event-Related Potentials and the Additive-Factors Method." *Journal of Cognitive Neuroscience* 8 (1): 47–68. doi:10.1162/jocn.1996.8.1.47.
- Dehaene, S, N Tzourio, V Frak, L Raynaud, L Cohen, J Mehler, and B Mazoyer. 1996. "Cerebral

- Activations during Number Multiplication and Comparison: A PET Study." *Neuropsychologia* 34 (11): 1097–1106. <http://www.ncbi.nlm.nih.gov/pubmed/8904747>.
- Dehaene, Stanislas. 1997. *The Number Sense: How the Mind Creates Mathematics*. First Edit. New York, USA: Oxford University Press.
- Dehaene, Stanislas, S. Bossini, and P. Giraux. 1993. "The Mental Representation of Parity and Number Magnitude." *Journal of Experimental Psychology. General*. doi:10.1037/0096-3445.122.3.371.
- Diester, Ilka, and Andreas Nieder. 2007. "Semantic Associations between Signs and Numerical Categories in the Prefrontal Cortex" 5 (11). doi:10.1371/journal.pbio.0050294.
- . 2008. "Complementary Contributions of Prefrontal Neuron Classes in Abstract Numerical Categorization." *Journal of Neuroscience* 28 (31): 7737–47. doi:10.1523/JNEUROSCI.1347-08.2008.
- Ditz, Helen M, and Andreas Nieder. 2015. "Neurons Selective to the Number of Visual Items in the Corvid Songbird Endbrain." *Proceedings of the National Academy of Sciences of the United States of America* 112 (25): 7827–32. doi:10.1073/pnas.1504245112.
- . 2016. "Numerosity Representations in Crows Obey the Weber-Fechner Law." *Proceedings. Biological Sciences / The Royal Society* 283 (1827). doi:10.1098/rspb.2016.0083.
- Eiselt, Anne-Kathrin, and Andreas Nieder. 2013. "Representation of Abstract Quantitative Rules Applied to Spatial and Numerical Magnitudes in Primate Prefrontal Cortex." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 33 (17): 7526–34. doi:10.1523/JNEUROSCI.5827-12.2013.
- Engel, Tatiana A., Warasinee Chaisangmongkon, David J. Freedman, and Xiao-Jing Wang. 2015. "Choice-Related Activity Fluctuations Underlie Learning of Neuronal Category Representation." *Nature Communications* 6 (March). Nature Research: 6454. doi:10.1038/ncomms7454.
- Fias, Wim, Johan Lauwereyns, and Jan Lammertyn. 2001. "Irrelevant Digits Affect Feature-Based Attention Depending on the Overlap of Neural Circuits." *Cognitive Brain Research* 12: 415–23. www.elsevier.com.
- Fischer, Martin H, and Julia Rottmann. 2005. "Do Negative Numbers Have a Place on the Mental Number Line?" *Psychology Science* 47 (1): 22–32.
- Gelman, Rochel, and C. R. Gallistel. 1978. *The Child's Understanding of Number*. January 19. Harvard University Press.
- Gerstmann, Josef. 1940. "Syndrome of Finger Agnosia, Disorientation for Right and Left Agraphia and Acalculia." *Archives of Neurology & Psychiatry* 44 (2): 398–408. doi:10.1001/archneurpsyc.1940.02280080158009.
- Harvey, B M, B P Klein, N Petridou, and S O Dumoulin. 2013. "Topographic Representation of Numerosity in the Human Parietal Cortex." *Science (New York, N.Y.)* 341 (6150): 1123–26. doi:10.1126/science.1239052.
- Henschen, S. E. 1919. "Über Sprach-, Musik- Und Rechenmechanismen Und Ihre Lokalisationen Im Großhirn." *Zeitschrift Für Die Gesamte Neurologie Und Psychiatrie* 52 (1). Springer-Verlag: 273–98. doi:10.1007/BF02872428.

- Isaacs, E B, C J Edmonds, A Lucas, and D G Gadian. 2001. "Calculation Difficulties in Children of Very Low Birthweight: A Neural Correlate." *Brain* 124 (9): 1701–7. <http://www.ncbi.nlm.nih.gov/pubmed/11522573>.
- Izard, Véronique, and Stanislas Dehaene. 2008. "Calibrating the Mental Number Line." *Cognition* 106 (3): 1221–47. doi:10.1016/j.cognition.2007.06.004.
- Jacob, Simon Nikolas, and Andreas Nieder. 2014. "Complementary Roles for Primate Frontal and Parietal Cortex in Guarding Working Memory from Distractor Stimuli." *Neuron* 83 (1): 226–37. doi:10.1016/j.neuron.2014.05.009.
- Kahn, H J, and H A Whitaker. 1991. "Acalculia: An Historical Review of Localization." *Brain and Cognition* 17 (2): 102–15. <http://www.ncbi.nlm.nih.gov/pubmed/1799448>.
- Lennert, Therese, and Julio Martinez-Trujillo. 2011. "Strength of Response Suppression to Distracter Stimuli Determines Attentional-Filtering Performance in Primate Prefrontal Neurons." *Neuron* 70 (1): 141–52. doi:10.1016/j.neuron.2011.02.041.
- Levy, L M, I L Reis, and J Grafman. 1999. "Metabolic Abnormalities Detected by 1H-MRS in Dyscalculia and Dysgraphia." *Neurology* 53 (3): 639–41. <http://www.ncbi.nlm.nih.gov/pubmed/10449137>.
- Mante, Valerio, David Sussillo, Krishna V Shenoy, and William T Newsome. 2013. "Context-Dependent Computation by Recurrent Dynamics in Prefrontal Cortex." *Nature* 503 (7474): 78–84. doi:10.1038/nature12742.
- Merritt, Dustin J, and Elizabeth M Brannon. 2013. "Nothing to It: Precursors to a Zero Concept in Preschoolers." *Behavioural Processes* 93 (February). Elsevier B.V.: 91–97. doi:10.1016/j.beproc.2012.11.001.
- Merritt, Dustin J, Rosa Rugani, and Elizabeth M Brannon. 2009. "Empty Sets as Part of the Numerical Continuum: Conceptual Precursors to the Zero Concept in Rhesus Monkeys." *Journal of Experimental Psychology: General* 138 (2): 258–69. doi:10.1037/a0015231.Empty.
- Merten, Katharina, and Andreas Nieder. 2012. "Active Encoding of Decisions about Stimulus Absence in Primate Prefrontal Cortex Neurons." *Proceedings of the National Academy of Sciences of the United States of America* 109 (16): 6289–94. doi:10.1073/pnas.1121084109.
- . 2013. "Comparison of Abstract Decision Encoding in the Monkey Prefrontal Cortex, the Presupplementary, and Cingulate Motor Areas." *Journal of Neurophysiology* 110 (1): 19–32. doi:10.1152/jn.00686.2012.
- Nieder, Andreas. 2005. "Counting on Neurons: The Neurobiology of Numerical Competence." *Nature Reviews. Neuroscience* 6 (3): 177–90. doi:10.1038/nrn1626.
- . 2012. "Supramodal Numerosity Selectivity of Neurons in Primate Prefrontal and Posterior Parietal Cortices." *Proceedings of the National Academy of Sciences* 109 (29): 11860–65. doi:10.1073/pnas.1204580109/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1204580109.
- Nieder, Andreas, and Stanislas Dehaene. 2009. "Representation of Number in the Brain." *Annual Review of Neuroscience* 32: 185–208. doi:10.1146/annurev.neuro.051508.135550.
- Nieder, Andreas, Ilka Diester, and Oana Tudusciuc. 2006. "Temporal and Spatial Enumeration

- Processes in the Primate Parietal Cortex." *Science* 313 (5792): 1431–35. doi:10.1126/science.1130308.
- Nieder, Andreas, David J Freedman, and Earl K Miller. 2002. "Representation of the Quantity of Visual Items in the Primate Prefrontal Cortex." *Science (New York, N. Y.)* 297 (5587): 1708–11. doi:10.1126/science.1072493.
- Nieder, Andreas, and Katharina Merten. 2007. "A Labeled-Line Code for Small and Large Numerosities in the Monkey Prefrontal Cortex." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 27 (22): 5986–93. doi:10.1523/JNEUROSCI.1056-07.2007.
- Nieder, Andreas, and Earl K Miller. 2003. "Coding of Cognitive Magnitude: Compressed Scaling of Numerical Information in the Primate Prefrontal Cortex." *Neuron* 37 (1): 149–57. <http://www.ncbi.nlm.nih.gov/pubmed/12526780>.
- . 2004. "A Parieto-Frontal Network for Visual Numerical Information in the Monkey." *Proceedings of the National Academy of Sciences of the United States of America* 101 (19): 7457–62. doi:10.1073/pnas.0402239101.
- Nuerk, Hans-Christoph, Wiebke Iversen, and Klaus Willmes. 2004. "Notational Modulation of the SNARC and the MARC (Linguistic Markedness of Response Codes) Effect." *The Quarterly Journal of Experimental Psychology. A, Human Experimental Psychology* 57 (5): 835–63. doi:10.1080/02724980343000512.
- Okuyama, Sumito, Toshinobu Kuki, and Hajime Mushiaki. 2015. "Representation of the Numerosity 'Zero' in the Parietal Cortex of the Monkey." *Scientific Reports* 5 (January). Nature Publishing Group: 10059. doi:10.1038/srep10059.
- Olthof, A, C M Iden, and W A Roberts. 1997. "Judgements of Ordinality and Summation of Number Symbols by Squirrel Monkeys (*Saimiri Sciureus*)." *Journal of Experimental Psychology. Animal Behavior Processes* 23 (3): 325–39. <http://www.ncbi.nlm.nih.gov/pubmed/9206027>.
- Park, J., N. K. DeWind, M. G. Woldorff, and E. M. Brannon. 2015. "Rapid and Direct Encoding of Numerosity in the Visual Stream." *Cerebral Cortex*, 1–16. doi:10.1093/cercor/bhv017.
- Pepperberg, Irene M. 1988. "Comprehension Of 'absence' by an African Grey Parrot : Learning with Respect to Question of Same/different." *Journal of the Experimental Analysis of Behavior* 50 (3): 553–64.
- . 2006. "Grey Parrot (*Psittacus Erithacus*) Numerical Abilities: Addition and Further Experiments on a Zero-like Concept." *Journal of Comparative Psychology* 120 (1): 1–11. doi:10.1037/0735-7036.120.1.1.
- Pepperberg, Irene M, and Jesse D Gordon. 2005. "Number Comprehension by a Grey Parrot (*Psittacus Erithacus*), Including a Zero-Like Concept." *Journal of Comparative Psychology* 119 (2): 197–209. doi:10.1037/0735-7036.119.2.197.
- Piazza, Manuela, Véronique Izard, Philippe Pinel, Denis Le Bihan, and Stanislas Dehaene. 2004. "Tuning Curves for Approximate Numerosity in the Human Intraparietal Sulcus." *Neuron* 44 (3): 547–55. doi:10.1016/j.neuron.2004.10.014.
- Piazza, Manuela, Andrea Mechelli, Cathy J Price, and Brian Butterworth. 2006. "Exact and Approximate Judgements of Visual and Auditory Numerosity: An fMRI Study." *Brain Research* 1106 (1): 177–88. doi:10.1016/j.brainres.2006.05.104.

- Piazza, Manuela, Philippe Pinel, Denis Le Bihan, and Stanislas Dehaene. 2007. "A Magnitude Code Common to Numerosities and Number Symbols in Human Intraparietal Cortex." *Neuron* 53 (2): 293–305. doi:10.1016/j.neuron.2006.11.022.
- Pinhas, Michal, and Joseph Tzelgov. 2012. "Expanding on the Mental Number Line: Zero Is Perceived as The 'smallest'." *Journal of Experimental Psychology. Learning, Memory, and Cognition* 38 (5): 1187–1205. doi:10.1037/a0027390.
- Raposo, David, Matthew T Kaufman, and Anne K Churchland. 2014. "A Category-Free Neural Population Supports Evolving Demands during Decision-Making." *Nature Neuroscience* 17 (12): 1784–92. doi:10.1038/nn.3865.
- Roitman, Jamie D, Elizabeth M Brannon, and Michael L Platt. 2012. "Representation of Numerosity in Posterior Parietal Cortex." *Frontiers in Integrative Neuroscience* 6 (May): 25. doi:10.3389/fnint.2012.00025.
- Roland, P E, and L Friberg. 1985. "Localization of Cortical Areas Activated by Thinking." *Journal of Neurophysiology* 53 (5): 1219–43. <http://www.ncbi.nlm.nih.gov/pubmed/3998807>.
- Rumbaugh, Duane M., Sue Savage-Rumbaugh, and Mark T. Hegel. 1987. "Summation in the Chimpanzee (Pan Troglodytes)." *Journal of Experimental Psychology: Animal Behavior Processes* 13 (2). American Psychological Association: 107–15. doi:10.1037/0097-7403.13.2.107.
- Seife, Charles. 2000. *Zero: The Biography of a Dangerous Idea*. Penguin Books.
- Shallice, T, and M E Evans. 1978. "The Involvement of the Frontal Lobes in Cognitive Estimation." *Cortex* 14 (2): 294–303. <http://www.ncbi.nlm.nih.gov/pubmed/679710>.
- Shepard, Roger, Dan W Kilpatric, and James P Cunningham. 1975. "The Internal Representation of Numbers." *Cognitive Psychology* 7 (1): 82–138. doi:10.1016/0010-0285(75)90006-7.
- Temple, E, and M I Posner. 1998. "Brain Mechanisms of Quantity Are Similar in 5-Year-Old Children and Adults." *Proceedings of the National Academy of Sciences of the United States of America* 95 (13): 7836–41. <http://www.ncbi.nlm.nih.gov/pubmed/9636237>.
- Tuduscic, Oana, and Andreas Nieder. 2007. "Neuronal Population Coding of Continuous and Discrete Quantity in the Primate Posterior Parietal Cortex." *Proceedings of the National Academy of Sciences of the United States of America* 104 (36): 14513–18. doi:10.1073/pnas.0705495104.
- Vallentin, Daniela, Sylvia Bongard, and Andreas Nieder. 2012. "Numerical Rule Coding in the Prefrontal, Premotor, and Posterior Parietal Cortices of Macaques." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 32 (19): 6621–30. doi:10.1523/JNEUROSCI.5071-11.2012.
- Vallentin, Daniela, and Andreas Nieder. 2008. "Behavioral and Prefrontal Representation of Spatial Proportions in the Monkey." *Current Biology*. Vol. 18. doi:10.1016/j.cub.2008.08.042.
- Viswanathan, Pooja, and Andreas Nieder. 2013. "Neuronal Correlates of a Visual 'sense of Number' in Primate Parietal and Prefrontal Cortices." *Proceedings of the National Academy of Sciences of the United States of America* 110 (27): 11187–92. doi:10.1073/pnas.1308141110.
- Wellman, Henry M., and Kevin F. Miller. 1986. "Thinking about Nothing: Development of

Concepts of Zero." *British Journal of Developmental Psychology* 4 (1): 31–42.
doi:10.1111/j.2044-835X.1986.tb00995.x.

Wheeler, Margariete, and Issa Feghali. 1983. "Much Ado about Nothing: Preservice Elementary School Teachers' Concept of Zero." *Journal for Research in Mathematics Education* 14 (3): 147–55.

Wynn, Karen. 1998. "Psychological Foundations of Number: Numerical Competence in Human Infants." *Trends in Cognitive Sciences* 2 (8). Elsevier: 296–303. doi:10.1016/S1364-6613(98)01203-0.

Wynn, Karen, and Wen-Chi Chiang. 1998. "Limits to Infants' Knowledge of Objects: The Case of Magical Appereance." *Psychological Science* 9 (6): 448–55.

Yu, Byron M, John P Cunningham, Gopal Santhanam, Si Ryu, Kv Shenoy, and M Sahani. 2009. "Gaussian-Process Factor Analysis for Low-Dimensional Single-Trial Analysis of Neural Population Activity." *Journal of Neurophysiology* 102 (1): 614–35.
doi:10.1152/jn.90941.2008.

11. Acknowledgements

Thanks to my parents, **Catalina y Sergio**, always and forever.

In the highs I think of you, and remember where I come from.

In the lows I think of you, and realize the long way I have gone through.

Thanks to **Juan**, for his company and support in those first German winters.

Thanks to those long-distance, but close-at-heart friends: **Alma, Alejandra, Arturo, Juan Carlos, Israel**.

Finally, thanks to those new friends who shared their lives with me in these years: **Pooja, David, Luis, Mafer, Ina, Erica**.

Thanks to my advisor, **Andreas Nieder**, for giving me a chance in science.

Thanks to my lab mates (**Pooja, Ina, Felix, Max, Torben, Nataljia**) for making my stay in science a pleasant enterprise.