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An abstract of
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Abstract

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Numerosity, or number representation without use of symbols, is a highly conserved across a variety of animals. Numerosity allows for quick, imprecise quantity judgements across stimulus modalities. Although number representation in humans and non-human primates has been well-studied, numerosity studies in other animals have only utilized behavioral paradigms. Particularly in dogs, these past behavioral tests have not provided strong evidence for innate number representation. The present study investigated neural activation in the canine cortex in response to changes in numerosity using awake, unrestrained fMRI. Eleven awake, unrestrained dogs passively viewed alternating dot arrays of 2 vs 10, 4 vs 8, or 6 vs 6 dots in a block fMRI design. After preprocessing, the data were split into two unique datasets to localize a region and test for a linear response to the ratios of 1, 2, or 5. In eight of the eleven dogs, we identified regions in the parietal cortex that were differentially active to the modulated effect of numerosity, which were replicable in an independent dataset at $p = 0.04$. These regions, although variable in location, were consistent with previous findings that had implicated the parietal cortex in encoding numerosity. To our knowledge, this study is the first to provide evidence of an untrained canine ability to discriminate changes in numerosity.

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Introduction

Defining Numerosity

Although number is commonly linked to the human-exclusive skill of mathematics, numerosity is shared across many species. Numerosity is the ability to create mental representations of number without relying on the use of symbols (Gross 2007). Therefore, possessing a sense of numerosity allows for an individual to briefly look at two sets of items and judge which set possesses a greater quantity. Numerosity is distinctive from counting in that counting is hypothesized to encompass an active encoding process in which attention must be diverted to every object (Ansari et al. 2007). In contrast, numerosity allows for a quick, but imprecise perception of number (Ansari et al. 2007).

Numerosity is non-symbolic in that individuals can extract numerosity from abstract stimuli, such as dots, which do not possess a particular meaning or association (Leibovich and Henik 2013 & Mock et al. 2018 & Liu et al. 2017). Humans are also capable of symbolic number representation, which is evident in the ability to associate number words, Roman numerals, and Arabic numerals with a particular value (Furman and Rubinsten 2012, Bialystok 1992, Pica et al. 2004). Because symbolic number is highly linked to language and culture, this number representation system is not found across species. Even among humans, symbolic number representations can vary drastically, as evidenced by one study in an indigenous group living in the Amazon (Pica et al. 2004). The Munduruku, who lack words expressing numbers greater than five, were unable to count or perform addition or subtraction when presented with large numbers (Pica et al. 2004).

In contrast, numerosity discrimination of dot arrays has been observed in infants as young as six months of age, which provides evidence for pre-literate, non-symbolic encoding of number

(Starr et al. 2013). Infants are also capable of discriminating number in a set of auditory tones (Cordes and Brannon 2009). Non-human primates, when tested in match-to-sample paradigms, have also been able to match number of auditory tones with the number of visual objects (Nieder 2012). Furthermore, the Munduruku, who lack symbolic number representation, were capable of differentiating numerosity changes in dot arrays with a similar accuracy to native French speakers that were also tested (Pica et al. 2004).

In summary, numerosity allows for a quick, imprecise representation of number and results in quantity discrimination across stimulus modalities.

Detecting Numerical Change

The ability to differentiate changes in numerosity are subject to Weber's law, which states that the ability to detect a difference in a stimulus is proportional to the stimulus magnitude (Ditz and Nieder 2016). As the ratio of the number of two stimuli increases, the accuracy of detecting these changes increases, which is also referred to as the numerical distance effect (Ditz and Nieder 2016). The numerical distance effect has been demonstrated not only in infants, but also in other primates and non-human primates (Cordes and Brannon 2009, Lipton and Spelke 2004, Ansari et al. 2007, Nieder 2012). Generally, as long as two sets of objects differ by a minimum ratio of two to one, most animals are capable of discriminating number.

Proposed Number Systems

Currently, the two proposed mechanisms for numerosity representation in the brain are the object file system and the approximate number system (ANS). In the object file system, each item is encoded as a specific object, whereas in the approximate number system, the numerosity of the stimuli is encoded as analog magnitude (Hyde 2011, Cordes and Brannon 2009). As a result, the approximate number system is also referred to as the analog magnitude system.

Generally, the ANS is considered to be more imprecise than the object file system, particularly as numerosity increases in absolute magnitude (Hyde 2011).

Although there are two distinct systems for numerosity, this does not exclude the possibility of coexistence within an individual. The object file system can account for a maximum of three to four items and is highly precise (Hyde 2011). This rapid encoding of a limited number of objects is often referred to as subitizing (Ansari et al. 2007). In contrast, the analog magnitude system is considered imprecise because it can only encode for approximate magnitude and is dependent on the ratio of the quantities. In accordance with Weber's law, if the ratios of the two quantities are not sufficiently large, then ability to discriminate differences decreases (Cordes and Brannon 2009). The analog magnitude system is usually associated with the process of estimation, which is defined as approximation of numerosity under conditions where subitizing and counting are not possible (Ansari et al. 2007).

Because of these differences in accuracy, it has been proposed that the object file system may be utilized primarily for small numerosity changes whereas the analog magnitude system is better suited for large numerosity changes (Hyde 2011). For instance, Cordes and Brannon (2009) reported that infants failed to discriminate between two sets of dot arrays when one stimulus contained less than three items and the other contained greater than four, even when the task was presented with favorable ratios. Cordes and Brannon concluded that these difficulties in discriminating between small and large sets may be due to the presence of both object file and analog magnitude systems in infants. If infants possess both number representation systems and utilize them for distinct purposes, then the small and large sets may be incomparable. Cordes and Brannon's conclusions are further supported by findings of anatomically distinct regions that respond differentially to large and small numerosity. In humans, neurons in the right temporal-parietal junction and left parietal regions were differentially active to smaller numbers whereas the right intraparietal regions were differentially active to larger numbers (Hyde and Spelke 2009). Furthermore, localization within the intraparietal sulcus (IPS) was reported by Xu (2009), in which the inferior regions of the IPS respond to object number whereas the superior IPS responds to differences in the identity of the object itself.

Evidence for Numerosity

To test for numerosity in animals, various paradigms have been used that not only control for other non-numerical variables but also preclude the ability to count (Piazza and Izard 2009), which I describe below…

a. Human Studies

Evidence of non-symbolic number representation has been observed in both infants and adults. After habituation to a series of sounds or dot arrays, infants focused on the presentation of a novel numerosity for a longer period of time, suggesting that they were able to discriminate number in both auditory and visual stimuli (Cordes and Brannon 2009). Lipton and Spelke (2004) also reached similar conclusions about the pre-literate ability to discriminate number using the same paradigm. Furthermore, the ratio required for proper number discrimination decreases in human infants over time, as nine-month old infants were capable of discriminating between 8 vs 12 auditory and visual stimuli, but six-month old infants were not (Lipton and Spelke 2004).

Within human neuroimaging studies, the primary focus has been on the locations in the brain that encode numerosity. In one study, dot arrays were sequentially presented within a block-fMRI design to identify regions involved with small- and large-number processing (Ansari et al. 2007). For each trial, participants focused on a cross while the experimenters quickly presented two dot arrays that varied in number (Ansari et al. 2007). Following the second array, participants determined whether the first dot array contained a greater, lesser, or equal number of dots than the second dot array. Within the right temporo-parietal junction, there was greater activation for small numbers relative to large numbers, with an average percent BOLD signal increase of 0.25% (Ansari et al. 2007). In contrast, activation to large numbers appeared to be suppressed in this same region, with a decrease in average percent BOLD signal of 0.10% (Ansari et al. 2007). Upon further analysis, there was greater activation to large relative to small numbers in the calcarine sulcus and parieto-occipital sulcus, suggesting that numerical processing of large and small numbers are localized to different regions of the brain (Ansari et al. 2007). Therefore, these data provide evidence for the presence of both object file and approximate number systems.

Other neuroimaging studies have implicated the posterior parietal cortex in numerosity encoding mechanisms, with a particular emphasis on the intraparietal sulcus (Harvey et al. 2015, Roitman et al. 2012). Although the IPS has been shown to contain neurons that encode numerosity, other studies suggest that the role of the IPS is not specific to numerosity as it also encodes for other stimuli characteristics such as color comparisons and object size (Piazza et al. 2004 & Shuman and Kanwisher 2004, Harvey et al. 2015). Overall, the results of the human studies suggest that number representation may be primarily associated with the parietal cortex, although the exact regions may vary depending on both the task and the subjects themselves.

b. Nonhuman Primate Studies

In non-human primates, some studies have utilized match-to-sample tasks as a method to test for numerosity. In one study, while two rhesus macaque monkeys matched auditory and visual stimuli in number, single-cell recordings revealed that the ventral intraparietal sulcus

(VIP) encoded numerosity of both modalities (Nieder 2012). Furthermore, these monkeys had neurons in the prefrontal cortex (PFC) tuned to numerosities of one to four, which means that these neurons tended to reach peak activity in response to a particular cardinal value, irrespective of modality (Nieder 2012).

Another study implicated the lateral intraparietal (LIP) region of rhesus macaque monkeys in number representation (Roitman et al. 2004). Single neurons within this region encoded all of the numerosities presented, from 2 to 32. However, LIP neurons did not encode cardinal values like the neurons found in posterior parietal cortex and prefrontal cortex in Nieder (2012). Rather, LIP neurons represented numerosity in a graded fashion, which means that as number increased or decreased, certain neural populations increased or decreased in activity. In other words, LIP neurons preferentially responded to the total number of dots presented, rather than preferentially activating to a particular numerical value. These findings suggest a potential intermediate step for number-encoding, in which LIP contains “accumulator” or “summation” neurons that integrate information prior to the actual encoding of numeric value. Another study proposed that these accumulator neurons represent either large or small numbers by variations in magnitude of activation, such that larger numbers result in greater activity (Verguts and Fias 2004). Once this information has been encoded, separate numerosity-specific neurons then encode a specific estimated cardinal value depending on degree of activation in accumulator neurons. This model of number-encoding proposed by Verguts and Fias suggests that a graded response should be encoded in accumulator neurons whereas a more refined encoding of cardinal value should be observed in numerosity-specific neurons, which is in accordance with the findings of Roitman et al (2004).

As a result of these primate studies, numerosity-encoding regions are primarily within the parietal cortex, much like in humans, suggesting that this region may have been evolutionarily conserved for number discrimination.

c. Non-Primate and Domestic Dog Studies

Although there is evidence for the conservation of numerical processing across many species, such as pigeons and salamanders, there is debate over whether domestic dogs possess numerical sensitivity (Emmerton and Renner 2006; Uller et al. 2003). In one study, a food choice paradigm was tested in thirteen domestic dogs (Petrazzini and Wynne 2016). These dogs were trained to select between two sets of food differing in either numerosity or total volume. Dogs failed to discriminate on the basis of numerosity when the number of food items was incongruent with total volume (Petrazzini and Wynne 2016). That is, dogs significantly preferred the set with the smaller number of items and a larger volume of food, suggesting that dogs relied on non-numerical cues in this particular paradigm (Petrazzini and Wynne 2016).

In another food choice paradigm, twenty-seven dogs were trained to observe the sequential dropping of food into two bowls, in which the number of food items varied numerically (MacPherson and Roberts 2013). Among the conditions tested of 0 vs 1, 1 vs 4, 1 vs 3, 1 vs 2, 2 vs 4, 2 vs 3, and 3 vs 4, dogs were only successful at selecting the higher quantity in the 0 vs 1 condition, with a collective success rate of 74%. In all other conditions, dogs selected the higher number 60% of the time or less, but these results were not statistically different than chance (Macpherson and Roberts 2013). Within that same study, one dog was trained to discriminate the same ratios from the first experiment, although they tested the simultaneous presentation of the two quantities instead of the sequential presentation used in the previous paradigm. The experimenters also tested the subject with unfamiliar ratios. With the exception of the most difficult ratio of 2 vs 3, the dog selected the higher quantity with a success rate that was greater than chance (MacPherson and Roberts 2013). Within this study, the inconsistency of most dogs to select for numerical quantity suggests that extent of number discrimination may vary on an individual basis.

Numerosity-Specific Regions in the Brain

In humans and non-human primates, regions of the parietal cortex such as the temporal-parietal junction, intraparietal sulcus, and lateral intraparietal region have been shown to respond to changes in numerosity (Ansari et al. 2007, Harvey et al. 2015, Roitman et al. 2012, Nieder 2012, Roitman et al. 2004).

In addition to the parietal regions, the visual cortex has also been implicated in numerosity. Number has been hypothesized to be visual in some aspects (Burr and Ross 2008). In a study by Fornaciai et al. (2017,) visual event-related potentials (ERPs) were used to determine the role of early visual cortex in processing numerosity. ERPs reached the greatest amplitude as early as 75 ms post-stimulus presentation and later ERPs reached the greatest amplitude at around 200 ms (Fornaciai et al. 2017). They also found that neural responses were more sensitive to numerosity than to non-numerical factors at around 90 ms post-stimulus, but numeric sensitivity was not observed later in the time series (Fornaciai et al. 2017). Furthermore, during this period of numeric sensitivity, Fornaciai et al. (2017) reported an inversion of polarity, which they attributed to the folding pattern around the calcarine sulcus, which is the defining topographical landmark for primary visual cortex. As a result, they suggested that V2 and V3 of the primary visual cortex are a part of the early encoding-response to numerosity (Fornaciai et al. 2017).

Early visual regions are hypothesized to create an object-location map, allowing for neurons involved with later-numerosity processing to integrate this information and approximate number (Dehaene and Changeux 1993). The close relationship between object-location and numerosity encoding is also supported by the finding that object-size neurons and number-neurons in the parietal cortex tend to be found in similar regions within the parietal cortex (Harvey et al. 2015). These object size and numerosity regions were distinct enough such that maps created for activation to both stimuli did not cover identical spatial extent but did have small regions of overlap (Harvey et al. 2015). Further evidence for the role of visual cortex in the encoding of numerosity was provided by the finding that numerosity was also subject to adaptation, much like other primary visual properties such as color, size, and density (Burr and Ross 2008).

Purpose and Hypothesis

Despite the numerosity studies performed in a variety of animals, these studies have been limited in their ability to assess innate numerical capabilities. Particularly in behavioral studies, extensive training is usually required to obtain a desired response. In one study investigating numerical discrimination capabilities within a single trial setting, untrained rhesus monkeys were tested for their ability to select the larger quantity of food (Hauser et al. 2000). They were able to only discriminate between the ratios of 2 vs 1 and 3 vs 2 (Hauser et al. 2000). In contrast, trained rhesus monkeys are able to more accurately discern number, as shown in Nieder (2012). This suggests that numerical skill may be improved with both increasing practice and positive reinforcement (Uller et al. 2001).

An alternative hypothesis is that over repeated trials, subjects that were not previously attending to number may alter their strategies for quantity discrimination. This hypothesis may correlate with the “last resort” hypothesis of numerosity in which some animals may not use numerosity as the most salient cue in decision making, but may rely on non-numerical cues such as density and total surface area of objects. In canine food choice paradigms, for example, most dogs tended to rely on non-numerical factors such as total object size to make a decision (Petrazzini and Wynne 2016 & Macpherson and Roberts 2013). However, there is also the possibility that dogs may utilize number in other paradigms that have yet to be tested.

Studies that have attempted to test for innate numerosity have primarily been performed in primates. However, most of these studies were invasive due to use of electroencephalograms and single cell recordings (Nieder 2012 & Roitman et al. 2004). Therefore, there was some level of manipulation that was required which raises questions about whether or not these findings would hold true within a more natural setting.

As a result of these limitations, the purpose of this study was to use awake, unrestrained fMRI in domestic dogs to test for neural sensitivity to number without requiring prior training on a behavioral task. If dogs have a sense of numerosity, then differential neural activation is predicted to occur in parietal regions and, possibly, visual cortex.

Methods

Participants

Eleven pet dogs from the Atlanta area participated in the fMRI study. All dogs were desensitized to the scanner environment through behavior shaping and desensitization. Dogs were not trained on the task of viewing dot arrays that varied in numerosity prior to their scan sessions.

MRI Scan Acquisition

Dog training and fMRI protocol were consistent with the procedures previously used in awake dog fMRI (Berns et al. 2012, Berns et al. 2013). The scans were obtained using a Siemens 3T Trio MRI scanner. To obtain functional scans, a single-shot echo-planar imaging (EPI) sequence was used to acquire volumes of 22 sequential 2.5 mm slices with a 20% gap (TE=25 ms, TR = 1260 ms, flip angle = 70 degrees, 64x64 matrix, 2.5 mm in-plane voxel size, FOV=192 mm).

For each individual, a total of approximately 1300 to 2000 functional volumes were acquired over the course of two to five runs. Each run lasted approximately seven minutes, with short breaks in between. For each dog, the total scan session lasted for a maximum of forty minutes.

Experimental design

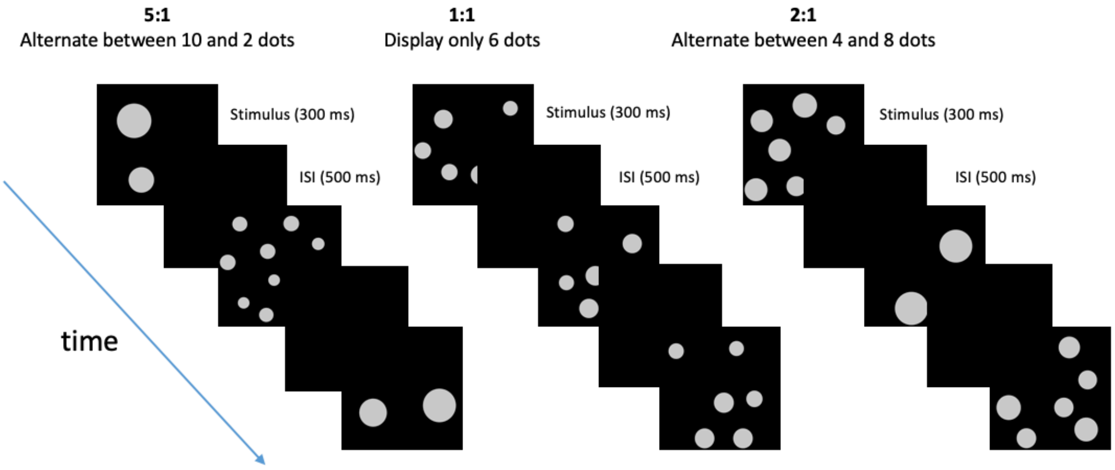

Figure 1. Dot array stimuli presented to dogs. The dot stimuli themselves were presented for only 300 ms. Between each stimulus, the interstimulus interval, a blank screen was displayed for 500 ms.

To test for ratio-dependent numerosity representation in the canine cortex, dot arrays that varied in number were passively presented to dogs within a block-fMRI design. The visual stimuli were projected onto a screen within the scanner bore, ensuring that the stimuli were clearly visible to dogs when they lied down.

The dot arrays were created using PsychoPy. On average, the dot arrays had constant total surface area and intensity. To control for total surface area while varying numerosity, the total area of dots was subdivided to create each condition. Within each block, the number of the dots varied between two values. For instance, in the three conditions of 5:1, 2:1, and constant, dogs viewed alternating dot arrays of 2 vs 10, 4 vs 8, or 6 vs 6 dots, respectively. Each block was presented for ten seconds in randomized order, but each block was presented an equal amount of times. Between blocks, treats were randomly distributed by the dog's handler to prevent undesired conditioning of stimuli and to maintain interest in the passive viewing task.

Using an MRI-compatible button box, stimulus onset times were recorded upon initiation of a new block. An experimenter standing in the rear of the scanner manually initiated each new condition to ensure that dogs were still in a suitable position within the scanner. Seven to ten seconds passed prior to presentation of the new block to allow for some rest periods.

Preprocessing

AFNI was used for both preprocessing and statistical analysis. For each individual dog, a reference volume that best represented his or her position for the duration of the scan was hand-selected. Using this reference volume, a mask was created to facilitate proper alignment of functional data to the brain. To account for subject motion, the aligned volumes underwent motion correction. Censoring was then performed with respect to both signal intensity and motion, in which voxels within the ROI with greater than 3% signal change and volumes with more than 1 mm of movement in any direction were flagged as spurious and censored from further analysis. The remaining volumes were then visually inspected for quality assurance. For most of the subjects, at least 50% of the total volumes were retained.

To improve signal-to-noise ratio (SNR), the data were then spatially smoothed with a 4 mm gaussian kernel. The data were normalized to percent signal change to ensure that all voxels are on a common relative scale.

Analysis

For each dog, a General Linear Model (GLM) was estimated for each voxel using the 3dDeconvolve function of AFNI. Because neural activation was predicted to vary with ratio, an amplitude-modulated response model (AM_2) was used. The task-related regressors were the response to dot array presentation and the linear response to the ratios of 1, 2, or 5. Because the experimental task lacked an explicit baseline, the parametric modulator allows for analysis of BOLD signal changes resulting from the changes in ratios. Non-task regressors included the six motion regressors obtained from motion correction.

Figure 2. Method of splitting data to localize and test for a linear response to the ratios of 1, 2, or 5.

Because of the high variability of significantly activated regions among dogs, each dog was analyzed independently of others. To ensure that activation was not due to noise, each dog's data were randomly split four-folds into localize and test datasets. The localize dataset contained 75% of the data whereas the test dataset contained the remaining 25%. Each dataset had at least two instances of each condition represented in each run. Furthermore, the difference of the average onset times of both datasets were less than five seconds apart to prevent disproportionately skewed data and minimize fatigue effects. As a final precaution, the experimental design matrices produced by each dataset were visually inspected to ensure a relatively even distribution of data across all runs.

As the name implies, the localize dataset was used to localize the most probable regions that corresponded to numerosity. The primary goal was to identify reasonably sized clusters with a reasonable voxel threshold within each dog. Given our small sample size, a conservative threshold for each voxel would likely result in many false negatives. Conversely, a liberal threshold would result in false-positives. In a previous fMRI study, participants performed a numerosity comparison task to determine whether or not patterns of brain activity were related to accuracy of numerosity discrimination (Lasne et al. 2018). Within this study, ROIs were defined with global visual cortex and parietal cortex masks in conjunction with voxels that were differentially active within each subject (Lasne et al. 2018). Lasne et al. selected the top 600 significantly activated voxels within each of the global masks to identify the most probable regions associated with numerosity. Our method was similar to this, although global masks were not used because they do not exist as a result of the wide variability among individual dogs. Furthermore, we could not use the top significantly activated voxels at the most conservative thresholds within each individual because these regions are likely to be spurious and due to noise, thus increasing the chance of a false-positive.

Therefore, as a compromise between the two extremes, the clusterize function of AFNI was used to create small clusters of approximately fifteen to forty voxels. The significance threshold at the voxel level was varied until one or two plausible clusters of positive voxel activation remained within each dog. These remaining clusters corresponded to the most significant voxels within a reasonably sized cluster. Then, using the test dataset for statistical inference, average beta values were extracted from regions identified by the localizer. The average linear change in BOLD signal in response to the modulated ratios of 1, 2 and 5 was then calculated for each dog. A t-test was performed across dogs testing the null-hypothesis that the average was zero. For visualization of locations, the functional ROIs identified for each individual dog were transformed into template space.

Results

Significant regions of activation in response to modulated numeric ratio was observed in eight out of eleven dogs (Figure 3). Laterality of these regions was observed with approximately equal frequency on either the left or right half of the brain. Despite the variability in where these regions occurred, most were located in the parietal cortex of the brain, although some regions appeared more parietotemporal.

The average beta values extracted from within these regions of interest ranged from -0.148 to 0.384 percent BOLD signal change/modulated ratio (Figure 4). Across the eight dogs, the mean value of the average beta values extracted from these regions was 0.131 percent BOLD signal change/modulated ratio, which was significantly different than zero (t = 2.05, df = 7, p = 0.04, one-sided.).

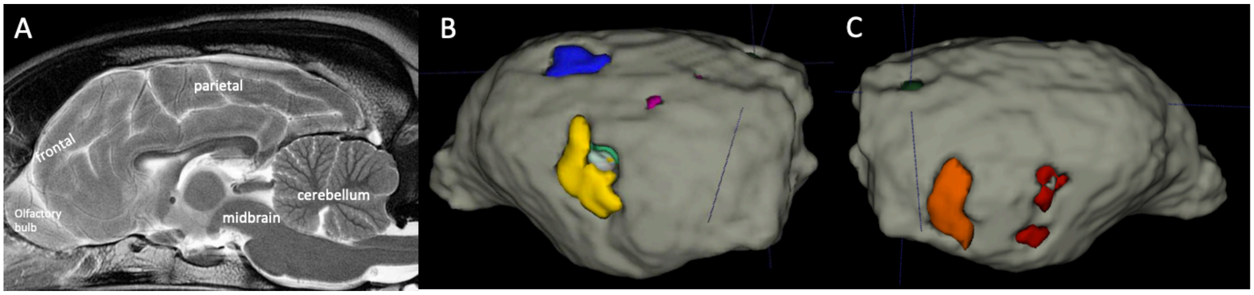

Figure 3. Individual functional ROIs mapped onto group space. **A)** Sagittal view of the canine brain, created by the University of Minnesota College of Veterinary Medicine as part of their MRI canine brain atlas (http://vanat.cvm.umn.edu/mriHeadAtlas/index.html). **B and C)** Functional ROIs located on the left **(B)** and right (**C)** sides of the brain. These functional ROIs of each dog were mapped on top of the digital canine brain atlas created by Datta et al. (2012). Each color represents a different dog, with the exception of the small area of overlap in B).

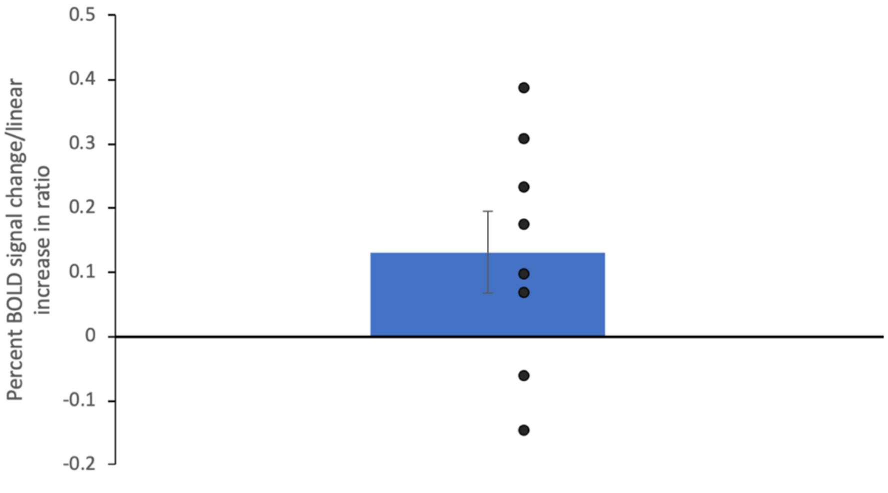

Figure 4. Distribution of average beta values extracted from each individual ROI using the test dataset overlaid on the average BOLD signal change/linear increase in ratio (t = 2.05, df = 7, p = 0.04, one-sided.).

Discussion

This study investigated sensitivity to changes in numerosity in the canine brain using awake fMRI. We hypothesized that if dogs had an innate ability to approximate number, regions of differential activation in the parietal cortex would be observed in response to dot arrays that varied in numerosity. In eight out of eleven dogs, identifiable regions of parietal cortex were differentially active to changes in the ratio of numerosity (Figure 3). Some of these regions are clustered in the anterior parietal cortex (Figure 3). Although the location of these regions were variable on an individual basis, the effect of numerosity was replicated in the test dataset at $p = 0.04$, which suggests that activation observed in these regions was not due to noise alone (Figure 4). Rather, activation represents the linear increase in BOLD signal in response to the ratios of 1, 2, and 5. Our results support the hypothesis that dogs have specific brain regions in the parietal cortex that respond to changes in numerosity.

Our findings of numerosity-specific regions in parietal cortex are consistent with previous human and non-human primate numerosity studies (Piazza et al. 2004, Nieder and Viswanathan 2013, Roitman et al. 2012, Nieder 2012, Eger et al. 2015). The majority of these studies have primarily focused on the role of intraparietal regions in encoding numerosity. For instance, some studies concluded that the horizontal segment of the IPS (hIPS) contains neurons that encode both symbolic and non-symbolic number (Dehaene et al. 2003 & Piazza et al. 2007). Another study found that the lateral intraparietal region contains neurons that encode individual numerosity values (Eger et al. 2015). Because of these previous studies that provide support for parietal cortex as encoding numerosity, we believe that we have also identified numerosity-specific regions in the canine brain.

Although the varying locations of numerosity-specific regions among individual dogs appears to contradict localization of numerosity to intraparietal regions in primates, other studies have implicated other regions that also respond to changes in numerosity. In particular, some studies have identified differential activation to numerosity in regions such as prefrontal cortex (Nieder 2012, Viswanathan and Nieder 2013, Ansari and Dhital 2006, Castelli et al. 2006), medial temporal lobe (Kutter et al. 2018), inferior temporal and middle occipital gyri (Shuman and Kanwisher 2004), and frontal gyri (Cantlon et al. 2006). This wide variability in numerosity-encoding regions suggests that these variations may be indicative of individual differences. In one study, whole brain analysis was used to identify whether differences in number approximation regions within the brain were correlated to mathematical ability in children (Kovas et al. 2009). Within this study, a wide distribution of brain regions with significant activation to number was observed, including the cerebellum, hippocampus, and dorsolateral prefrontal cortex (Kovas et al. 2009). Furthermore, variations in location of numerical sensitivity were correlated with differences in mathematical ability in children. High ability children had greater activation in cerebellum, left claustrum, and right calcarine sulcus (Kovas et al. 2009). Conversely, low ability children had greater activation in left lingual gyrus and right thalamic area (Kovas et al. 2009). These varying regions of activation in response to changes in number may be indicative of individual differences in numerical acuity. If variations in the locations of clusters are predictive of numerical competence, then this may suggest that the dogs within our study also vary in ability to detect changes in numerosity.

Numerosity-specific regions were observed bilaterally at the group level, although within each individual dog, these regions were lateralized. In part, this was a result of the method used to isolate these numerosity-specific clusters. Because we only selected the most significant cluster of voxels, it is possible that dogs have bilateral regions in the cortex that respond to changes in numerosity. However, these other regions may not been significant at the chosen threshold. Some primate studies report bilateral activation in both passive and (Cantlon et al. 2006, Piazza et al. 2004, Piazza et al. 2007) active numerosity discrimination tasks (Ansari and Dhital 2006, Castelli et al. 2006). Based on the current experimental design, we are unable to evaluate whether or not bilateral numerosity regions exists within dogs. However, other studies suggest that one side of the brain may be more capable of detecting changes in numerosity (Izard et al. 2004 & Harvey et al. 2015). In one study that reported bilateral activation in intraparietal regions, Weber fractions were calculated using both right and left intraparietal activation to provide a measure of ability to detect change in numerosity (Izard et al. 2004). The Weber fraction obtained from right intraparietal region was slightly greater ($w = 0.25$) compared to that of the left intraparietal region ($w = 0.17$), although this difference was not significant (Izard et al. 2004). These findings of different Weber fractions within the same test subjects suggest that perhaps one side of the brain is more capable of detecting numerical change than the other.

Other studies also provide support for lateralization of numerical acuity in the brain (Harvey et al. 2013, Dormal et al. 2010, Dormal et al. 2012, Piazza et al. 2006). In one study, dot arrays were presented in a block fMRI design in which numerosity was varied while controlling for confounding variables of total dot area, individual dot size, total dot circumference, dot density, and object shape (Harvey et al. 2013). Numerosity maps were constructed from small populations of neurons that displayed significant activation to changes in numerosity (Harvey et al. 2013). Harvey et al. concluded that because the left numerosity map was weaker and less consistent than the right numerosity map that numerosity is likely lateralized to the right side of the human brain (Harvey et al. 2013). Another study investigating the effects of transcranial magnetic stimulation (TMS) on numerical capabilities revealed that right IPS stimulation resulted in decreased accuracy in determining which quantity was larger whereas left IPS stimulation did not have a noticeable effect, which suggests that numerical acuity is primarily dominated by the right IPS (Dormal et al. 2012). In light of reported lateralization of numerical acuity, this may explain the laterality of numerosity-specific regions observed in the canine brain, in which one side may be more capable of detecting change in numerosity than the other.

Identification of a numerosity-specific region in the parietal cortex in eight out of the eleven dogs tested is significant in providing further insight on innate numerosity in domestic dogs. In a past behavioral study, thirteen dogs were unable to correctly select the higher quantity of food items when presented with incongruent volume and number (Petrazzini and Wynne 2016). On a group level, dogs preferred the food sets with higher total volume rather than those with higher number (Petrazzini and Wynne 2016). As a result, Petrazzini and Wynne failed to observe numerosity and concluded that food choice in dogs was controlled primarily by non-numerical cues. In another study, with the exception of one individual, most dogs were incapable of discriminating number in a food choice paradigm for any condition beside 0 vs 1 (Macpherson and Roberts 2013). As a result of these behavioral studies, the general consensus on numerosity in dogs was that while some dogs may possess a sense of number, numerosity may not be the most salient cue. Our results suggest that numerosity may be more innate in dogs than previously thought.

Our findings of numerosity in dogs is interesting, considering that dogs may no longer utilize numerical cues to the same extent as other wild animals. As a result of the long history of domestication and coevolution with humans, pet dogs are behaviorally different from their wild ancestors (Werhahn et al. 2016, Miklosi et al. 2003, Pendleton et al. 2018). For instance, one study investigated the differences between domestic dogs and wolves in their ability to respond to human gaze (Werhahn et al. 2016). While wolves displayed an ability to follow distant gazes, dogs only reacted when the gaze was directed toward them (Werhahn et al. 2016). An ability to react to gaze may provide an advantage in that environmental information can be quickly obtained. This differential gaze-following may be indicative of decreased salience of environmental cues when humans are present. Perhaps in dogs the ability to detect changes in numerosity was not conserved to the same extent as in wolves, as most pet dogs are fed consistently. In one study comparing the effects of ratio on number discrimination in dogs and wolves, food preferences in wolves were independent of ratio (Petrazzini and Wynne 2017). For all ratios tested (1 vs 3, 2 vs 4, 2 vs 3, and 3 vs 4), wolves selected the greater quantity of food with a success rate that was significantly different than chance (Petrazzini and Wynne 2017). In contrast, dogs only discriminated the higher quantity of food in the 1 vs 3 and 2 vs 4 condition (Petrazzini and Wynne 2017). The ability of wolves to select greater quantities in food regardless of ratio provides support that wolves may be more adept at discriminating number because they must hunt to obtain food. In contrast, the ability to detect changes in number may not be a selective pressure for dogs given the effects of domestication and artificial selection. However, through fMRI we were able to show that dogs possess innate numerosity discrimination.

One limitation in our study was the difficulty in comparing numerosity regions across dogs. Dogs have more variable brain anatomy than either humans or monkeys. Through artificial selection, the physical variation in dogs is unmatched by other species (Schoenebeck and Ostrander 2013). Dogs have been selected for traits to fulfill particular roles such as herding, hunting, or guarding, which has had significant implications on skull morphology. These differences in skull shape are correlated with differences in cerebral organization of dog brains.

Using high-field fMRI, the anterior end of the brain was observed to be rotated ventrally in dogs with reduced skull lengths (Schoenebeck and Ostrander 2013). In addition, the olfactory lobes in these brachycephalic breeds were ventrally located relative to other dogs (Schoenebeck and Ostrander 2013). While individual differences in brain anatomy also exist among humans and monkeys, these variations are less pronounced relative to domestic dogs.

Another limitation in our study was the difficulty in comparing the numerosity regions identified within the canine brain to regions of increased activation to numerosity in humans and monkeys. For example, the canine intraparietal region has yet to be identified. To overcome this limitation, perhaps a future study could attempt to define a region within the canine brain that is analogous to the intraparietal region, given that this region is known to contain neurons with other functions that are unrelated to numerosity. For instance, the lateral intraparietal region has also been shown to encode saccades (Goldberg et al. 2006). Perhaps in a future study, saccadic movements could be incorporated into the passive viewing task in order to functionally first functionally determine the canine equivalent of the lateral intraparietal region and then test for the effect of changes in numerosity. This method of functional localization was attempted in one study which investigated whether humans possessed regions that are analogous to macaque lateral and ventral intraparietal regions (LIP and VIP) that encode number (Eger et al. 2015). In this study, regions of the parietal cortex that were active to saccadic eye movements were identified as the human-equivalent of LIP. Parietal regions that were active to both dot motion and tactile stimulation were functionally defined as VIP. Using these regions of interest, number selectivity was tested through dot arrays. Eger et al. concluded that these regions also contain neurons tuned to individual numerosity like the LIP and VIP in macaques, thus providing evidence for analogy between humans and primates (Eger et al. 2015). In a future study, saccades could also be used to functionally localize a region which can be used to test for sensitivity to changes in numerosity and allow for better comparison of canines to primates.

Other limitations with our study involve the stimuli themselves. Because cumulative area of dots was controlled across all blocks, mean variation in element size between successive stimuli (10 vs 2 and 4 vs 8 dots) may be correlated with ratio. As a result, the average change in element size is greatest within the 5:1 condition. In order to ensure that the effects observed are truly due to the changes in numerosity alone, a future experiment could potentially test other ratios such as 2 vs 3 and 3 vs 4 to include mean variation in element size as an additional regressor in the GLM. Furthermore, because number representation in dogs was modeled linearly, including different ratios may result in a model with a better fit. Another limitation is the use of dots as visual stimuli. Although we were able to show an effect using dot arrays, it is unclear how dogs would respond to more natural stimuli, suggesting that a future study could include the use of more salient stimuli.

Our results provide evidence for an untrained numerosity representation system in domestic dogs. The ability of domestic dogs to detect changes in numerosity provides support for a conserved mechanism that a wide variety of animals can utilize for quantitative decisions. We were able to observe this numerosity effect through a passive viewing paradigm, which is significant because dogs are not naturally accustomed to viewing screens. Furthermore, because the dot stimuli are not salient to dogs, there were not high expectations for dogs to complete the task. In addition, we were able to observe an effect with a relatively small amount of data and without prior exposure to the viewing paradigm, which suggests that this ability to approximate number is innate and was observed with as little manipulation possible.

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