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Awake fMRI Reveals Mechanisms of Language Comprehension in Dogs

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Abstract

To what extent do dogs understand human language? At a basic level, lexical processing would require the differentiation of words from non-words, while semantic processing would further require the recognition of word meaning. To determine the level of language processing, we trained 12 dogs to retrieve two objects based on object names, then probed the neural basis for these auditory representations using awake-fMRI. As a control, we compared the neural response to pseudowords versus trained words, and novel objects versus trained objects during the fMRI scan. If dogs lexically processed the trained words, then a differential auditory response would be predicted to occur to trained words relative to pseudowords. Moreover, if dogs used semantic processing, there should be a difference in activation between the two trained words. In support of lexical processing, we found greater activation for pseudowords relative to trained words bilaterally in the parietotemporal cortex, but no activation difference between the two trained words. However, multivoxel pattern analysis (MVPA) revealed statistically significant clusters of informative voxels for the two trained words in the thalamus, left temporoparietal region, and left caudate nucleus. These results suggest a different mechanism of language comprehension than in humans. The dogs' greater activation for pseudowords in the parietotemporal cortex indicates an underlying bias for novelty rather than semantic processing of words as object-referents, while the MVPA results suggest a potential link between words/sounds and motor action.

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Introduction

Although dogs have a well-known ability to follow verbal commands, the degree to which dogs understand human language remains unknown. At a basic level, dogs can discriminate between words and arbitrary utterances [1,2], suggesting they have the capacity for lexical processing. Beyond lexical processing, a few dogs have even shown the potential for understanding the meaning of a large number of words, suggesting the potential for semantic processing [2,3]. For example, humans recognize that the word, "ball," is a word and that it has many potential meanings: spheres, sports equipment, and actions. Although the human-ascribed content of a word can refer to an action, a location, a person, food, or an object, this is not necessarily true for a dog. Apart from a few individuals, dogs may not depend on word processing as humans do, but instead rely on other cues to follow verbal commands, such as gaze [4], third-party interactions [5], gestures [6,7], emotional expression [8], and phonetic characteristics [6,9]. Thus, a dog's attention to words does not prove that they understand meaning, i.e. semantics.

In human studies, functional magnetic resonance imaging (fMRI) is often used to identify brain regions that support specific aspects of language. In general, the left hemisphere, including the temporal cortex and inferior frontal cortex, tends to support syntactic processes, or the arrangement of words, whereas the temporal and frontal areas across both hemispheres support semantic processes [10–14]. More specifically, lexical processing refers to the ability to identify a word compared to nonwords, typically pseudowords that are similar in the number of syllables and structure but do not have any meaning. Human studies have shown greater responses to words than pseudowords in the left posterior middle temporal and angular gyri, the rostral and caudal cingulate gyrus, the precuneus, and the right inferior temporal gyrus [15–17]. However, the type of pseudoword generated for the task can alter the direction of activation, such that pseudowords sounding similar to known words elicit greater activation than unique pseudowords in an adjacent temporal-parietal region to that which responds to known words [15].

In contrast to lexical processing, semantic processing involves a distinction between the meaning of words. FMRI research involving individual words and sentences has shown that semantic information is widely distributed across the brain, but regions in the left temporal and inferior parietal cortex show the highest activation and prediction accuracy for semantic content [18]. Recent fMRI studies have employed multivoxel pattern analysis (MVPA) to correlate the patterns of regional brain activity with the semantic features that compose a noun, and have further demonstrated that words with similar semantic content are represented in neighboring brain areas [10,19].

Part of the problem in studying language comprehension in dogs is the necessity of a behavioral response to demonstrate understanding. A common test for word comprehension in dogs assesses whether they use verbal referents to retrieve an object. Dogs can reliably retrieve an object based on a command combined with the name of the object, but this often requires months of training. Examples include Chaser, the border collie who learned over one thousand object-word pairings,

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and the border collie Rico, who demonstrated the ability to select a novel object among familiar objects based on a novel label [2,3,20]. Other studies examining word-learning in dogs have separated the noun from the verb in a given command, where the dog is commanded to "paw, touch or take" a named object. This training resulted in dogs with flexibility in their indication behavior depending on the verb/noun combination of the command [21,22]. Although it is tempting to attribute object retrieval skills to semantic processing, a dog's ability to retrieve an object following a verbal command does not preclude non-linguistic explanations like associative learning (the object and command are paired close together in time i.e. temporal contiguity), or learning by exclusion [23]. Investigation of language processing in dogs may therefore benefit from an approach that does not depend exclusively on a behavioral action.

Recent advances in awake neuroimaging in dogs have provided an avenue to investigate many aspects of canine cognition, including language processing. Since 2012, pet dogs have been trained using positive reinforcement to lie still during fMRI scans in order to explore a variety of aspects of canine cognition [24,25]. MRI scans have furthered our understanding of the dog's neural response to expected reward [26], identified specialized areas in the dog brain for processing faces [27,28], observed olfactory responses to human and dog odors [29], and linked prefrontal function to inhibitory control [30]. Recently, fMRI was used to show individual differences in dogs' ventral caudate activation during expectation of food or praise, indicating a dog's preference for one over the other [31]. FMRI has also been used to investigate auditory processing in canines. In an initial study, dogs listening to human and dog vocalizations through headphones showed activations within regions of the temporal and parietal cortex [32]. A follow-up study suggested a hemispheric bias for praise words versus neutral words, a finding that was interpreted as proof of semantic processing in dogs, although a subsequent correction in which left and right were reversed raised questions about the interpretability of this finding [33]. Thus, there is still great uncertainty over the extent to which dogs understand human language and the mechanisms that they employ to parse our utterances.

To examine language processing in dogs, we used fMRI to measure activity in dogs' brains in response to both trained words and pseudowords. Because there is a large literature on fMRI and language comprehension in humans, dogs' responses under similar paradigms can be compared to humans for evidence of either lexical or semantic processing. Prior to scanning, owners trained their dogs to select, or retrieve, two objects based on the objects' names. During the fMRI session, the owner spoke the names of the trained objects as well as pseudowords the dog had never heard. If dogs lexically process words like humans do, they should show differential activity in the parietal and temporal cortex in response to trained words relative to pseudowords [15–17]. Similarly, if dogs demonstrate semantic processing, then there should be a difference between the two trained words, as they should serve as referents for different objects. Because differential activity in the canine caudate nucleus has been used to compare social and food rewards, there should also be differential activity in the caudate in response to the trained words relative to the pseudowords, as the trained words had been associated with food and/or social

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praise [31]. Likewise, if dogs have an underlying preference for one trained object over the other, dogs would show a higher caudate response to the word associated with that object, or the object itself.

Methods

Ethics Statement

This study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The study was approved by the Emory University IACUC (Protocol DAR-2002879-091817BA), and all owners gave written consent for their dog's participation in the study.

Subjects

Subjects were pet dogs from the Atlanta community volunteered by their owners for fMRI training and experiments (Table 1). All dogs had previously completed one or more scans for the project and had demonstrated the ability to remain still during training and scanning [24,26,31].

Table 1. Dogs and their object names.

Dog	Breed	Age	Sex	Years with fMRI project	Object 1	Object 2
Caylin	Border Collie	8	Spayed F	4	Monkey	Blue
Eddie	Golden Retriever-Lab mix	6	Neutered M	2	Piggy	Monkey
Kady	Golden Retriever–Lab mix	7	Spayed F	4	Taffy	Yellow
Libby	Pit mix	11	Spayed F	4	Duck	Hedge Hog
Ninja	Australian Cattle dog- mix	2	Spayed F	1	Block	Monkey
Ohana	Golden Retriever	7	Spayed F	3	Blue	Star
Pearl	Golden Retriever	7	Spayed F	3	Duck	Elephant
Stella	Bouvier	6	Spayed F	3	Stick	Tuxy
Truffles	Pointer mix	12	Spayed F	2	Pig	Blue
Velcro	Viszla	8	Intact M	3	Rhino	Beach Ball
Zen	Golden Retriever– Lab mix	8	Neutered M	4	Teddy	Duck
Zula	Lab-Mastiff mix	4	Spayed F	1	Goldie	Bluebell

Dog's names, breed, age in years when undergoing scanning, sex, years participating in fMRI experiments, and training objects (S+) are listed

MRI Behavioral Training

All dogs in the current study participated in training for previous fMRI experiments, As described in previous experiments [24–26,31], each dog had participated in a training program involving behavior shaping, desensitization, habituation and behavior chaining to prepare for the loud noise and physical confines of the MRI bore inherent in fMRI studies.

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Word-Object Training

In the current experiment, dogs were trained to reliably fetch or select a trained object given the matching verbal name for the object. The dogs were trained by implementing the "Chaser Protocol" in which object names were used as verbal referents to retrieve a specific object [2]. To keep the task simple, each dog had a set of two objects, selected by the owner from home or from dog toys provided by the experimenters. One object had a soft texture, such as a stuffed animal, whereas the other was of a different texture such as rubber or squeaked, in order to be clearly discriminable (Fig. 1).

Each dog was trained by his or her owner at home, approximately 10 minutes per day, over 2 to 6 months, as well as at biweekly practices located at a dog training facility. Initial shaping involved the owner playing "tug" or "fetch" with her dog and one object while verbally reinforcing the name of the object. Later, the objects were placed at a distance (four feet on average) and the owner instructed the dog to "go get [object]" or "where is [object]?" or "[object]!" The dog was reinforced with food or praise (varied per dog) for retrieving or nosing the object. Next, the object was placed beside a novel object roughly two feet apart, at least 4 feet from the dog, and the command repeated. The dog was reinforced only for correctly selecting the trained object if it was her first selection. Otherwise, if the dog selected the wrong object, the owner made no remark and a new trial began. Regardless of the selection, objects were rearranged before each trial to limit learning by position. If the dog failed to approach an object, the trial was repeated. This training was repeated for each dog's second object against a different comparison object, to limit the possibility of learning by exclusion. Owners were instructed to train one object per day, alternating between objects every other day until they showed the ability to discriminate between the trained and novel object, at which point they progressed to discrimination training between the 2 trained objects.

Word-Object Discrimination Tests

Two weeks after progressing to two-object discrimination training, and every two weeks thereafter, each dog was tested on her ability to discriminate between the two trained objects. Discrimination between the two named objects was chosen as the measure of performance, as both objects had a similar history of reinforcement, and this precluded the possibility that performance was based on familiarity. Discrimination testing consisted of the observer placing both trained objects 2-3 feet apart, and at least 4 feet from the dog [34]. With the dog positioned next to the owner in the heel position, the owner gave the dog the command to "go get [object]" or "[object]!" The dog was reinforced only for correctly selecting the trained object if it was her first selection. If the dog selected the incorrect object, the owner made no remark. After each trial, the objects were rearranged and the test progressed to the next trial. A performance criterion to move forward to the MRI scan was set at 80% correct for at least one of the objects, with the other object at or above 50%.

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During training, owners were asked to report if their dog showed a preference for one object over the other. For the majority of the dogs, the preference was for the softer object of the two, and both the preferred word and the object were consistently labeled as word 1 and object 1. Though Zula passed the discrimination test, she was unable to complete the MRI scan and was excluded from the remainder of the study. Individuals varied on the amount of time needed to train both objects.

Scan Day Discrimination Test

Scan day tests were conducted in a neighboring room to the MRI room, and were typically conducted prior to the MRI scan. Test procedure was identical to the word-object discrimination test as described above, although the number of trials was increased from 10 to 12 trials if the dog failed to make a response during one or more trials.

fMRI Stimuli

The stimuli consisted of the two trained words and the corresponding objects. Pseudowords were included as a control condition. Pseudowords were matched to the group of trained words based on the number of syllables and bigram frequency where possible using a pseudoword generator [35] (Table 2). Phoneme substitution was necessary in some cases to ensure that trained words and pseudowords did not overlap at onset or coda. During the scan, pseudowords were followed by the presentation of novel objects with which the dogs had no previous experience. The novel objects included a bubble wand, Barbie doll, stuffed caterpillar, wooden train whistle, plastic gumball dispenser, yellow hat, watermelon seat cushion, Nerf ball launcher, etc.

Table 2. List of pseudowords per run.

Run 1	**Run 2**	**Run 3**
prang	cal	cloft
risnu	o gri	sowt
doba	ropp	bodmick
bobbu	prel	fons
zelve	thozz	stru

fMRI Experimental Design

As in previous studies, dogs were stationed in the magnet bore using custom chin rests. All words and objects were spoken by the dog's primary owner, who stood directly in front of the dog at the opening of the magnet bore. At the onset of each trial, a word was projected onto the surface of the scanner, directly above the owner's head. An experimenter stood next to the owner, out of view of the dog. The experimenter controlled the timing and presentation of the words to the owner via a four-button MRI-compatible button box (Fig. 2A).

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An event-based design was used, consisting of four trial types presented semi-randomly: congruent, incongruent, pseudoword, and reward. On congruent trials, the owner repeated a trained object's name five times, once per second. Words were repeated to ensure a robust hemodynamic response on each trial and spoken loudly to be heard above the scanner noise. After a variable 3 to 8 s delay, the dog was shown the corresponding object for 5s and was subsequently allowed to interact with the object. During incongruent trials, the owner repeated the name for a trained object as above, but following the delay period a novel object was presented instead of the correct object. In pseudoword trials, the owner repeated a pseudoword, and the delay was followed by a novel object. Reward trials were interspersed throughout each run, during which the owner rewarded the dog's continued down-stay with food. Trials were separated by a 6s inter-trial interval, and each dog received the same trial sequence (Figure 2B). Each of three runs consisted of 26 trials, for a total of 78 trials. The trial types included: 30 congruent, 15 incongruent, 15 pseudowords, and 18 food rewards.

Imaging

Scanning for the current experiment was conducted with a Siemens 3 T Trio whole-body scanner using procedures described previously [24,25]. During previous experiments, a T2-weighted structural image of the whole brain was acquired using a turbo spin-echo sequence (25-36 2mm slices, TR = 3940 ms, TE = 8.9 ms, flip angle = 131˚, 26 echo trains, 128 x 128 matrix, FOV = 192 mm). The functional scans used a single-shot echo-planar imaging (EPI) sequence to acquire volumes of 22 sequential 2.5 mm slices with a 20% gap (TE = 25 ms, TR = 1200 ms, flip angle = 70˚, 64 x 64 matrix, 3 mm in-plane voxel size, FOV = 192 mm). Slices were oriented dorsally to the dog's brain (coronal to the magnet, as in the sphinx position the dogs' heads were positioned 90 degrees from the prone human orientation) with the phase-encoding direction right-to-left. Sequential slices were used to minimize between-plane offsets from participant movement, while the 20% slice gap minimized the "crosstalk" that can occur with sequential scan sequences. Three runs of up to 700 functional volumes were acquired for each subject, with each run lasting 10 to 14 minutes.

Analysis

Preprocessing

Data preprocessing included motion correction, censoring and normalization using AFNI (NIH) and its associated functions. Two-pass, six-parameter affine motion correction was used with a hand-selected reference volume for each dog. Aggressive censoring (i.e., removing bad volumes from the fMRI time sequence) was used because dogs can move between trials, when interacting with the object, and when consuming rewards. Data were censored when estimated motion was greater than 1 mm displacement scan-to-scan and based on outlier voxel signal intensities. Smoothing, normalization, and motion correction parameters were identical to those described previously [31]. A high-resolution canine brain atlas [36] was used as the template space for

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individual spatial transformations. The Advanced Normalization Tools (ANTs) software was used to spatially transform the statistical maps of the contrasts of interest [37] to the template brain.

General Linear Model

Each subject's motion-corrected, censored, smoothed images were analyzed with a general linear model (GLM) for each voxel in the brain using 3dDeconvolve (part of the AFNI suite). Motion time courses generated through motion correction, and constant linear, quadratic, and cubic drift terms were included as nuisance regressors. The drift terms were included for each run to account for baseline shifts between runs as well as slow drifts unrelated to the experiment. Task related regressors were modeled using AFNI's dmUBLOCK function and were as follows: (1) spoken word 1; (2) spoken word 2; (3) spoken pseudowords; (4) presentation of congruent objects; (5) presentation of incongruent objects; and (6) presentation of novel objects. The object on which each dog performed best during the day of the MRI scan was labeled as word 1 and object 1 when creating the GLM regressors.

Contrasts of interest included: the pseudowords compared to trained words [pseudowords – (word1 + word2)/2], the difference between trained words [word1 – word2], the difference between trained objects [object1- object2], and presentation of incongruent objects compared to the presentation of novel objects [incongruent – novel].

Region of Interest Analysis

Because our main interest was in the dog's response to trained verbal words compared to pseudowords, all quantitative analyses based on the imaging results used activation values in the canine parietotemporal area previously observed to be responsive to vocalizations [32]. Anatomical ROIs of the right (3170 mm^3) and left parietotemporal regions (3361 mm^3), including auditory cortex, were drawn using the canine brain atlas [36] (Fig. 3). Mean beta values for the primary contrasts [pseudowords - words], [word1 - word2], and [object1 - object2] were extracted for each ROI. The mean contrast values for each ROI in each dog were then used to determine whether there was a significant difference between words and pseudowords, between trained words, and between trained objects, as well as whether there was any hemispheric difference. We used the mixed-model procedure in SPSS 24 (IBM) with fixed-effects for hemisphere, random effects for dog, identity covariance structure, and maximum-likelihood estimation. As habituation to the repeated words throughout the scan session was a possibility, we performed a second GLM that extracted beta values for each trial from the parietotemporal ROI for pseudowords and trained words (using the stim_times_IM function from AFNI's 3dDeconvolve suite). These trial-dependent beta values were compared using the mixed-model procedure as described above but including trial number as a covariate. Because any habituation would be expected to be nonlinear in time, we used ln(trial number) in the mixed-model.

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Spherical ROIs in the right and left caudate nuclei were drawn using the same canine brain atlas [31,36] (Fig. 3). Mean beta values for the contrasts [pseudowords - words], [word1 - word2], and [object1 - object2] were extracted for each ROI. Additionally, mean beta values for the contrast [novel-incongruent] were extracted to compare the presentations of novel objects across the two conditions. The mean contrast values for each ROI in each dog were then used in a mixed-effect linear model as described above to determine whether there was a significant difference between pseudowords, words, trained words, trained objects, novel objects and whether there was any hemispheric difference.

MVPA Analysis

As an exploratory analysis, we used multivoxel pattern analysis (MVPA) to identify potential cortical representations of word1 and word2 not captured in mean activation levels from the univariate GLM. First, each subject's unsmoothed, but motion-corrected functional data was input to a GLM that fit voxelwise beta values for each trial for word1 and word2 (using the stim_times_IM function from AFNI's 3dDeconvolve suite). Volumes were censored for motion and outlier count as above. Second, these trial-dependent beta values were then used as inputs to a whole-brain searchlight MVPA using PyMVPA2 [38]. Both linear support vector machine (SVM) and penalized logistic regression classifiers were examined and yielded qualitatively similar results. The final analysis used SVM because of its previously demonstrated robust performance [39,40]. An attributes file for each dog was generated from the GLM design matrix by coding all instances of word1 and word2. The classifier was then trained on the fMRI dataset for each dog using 2 runs and testing on the third using the NFoldPartitioner. For the searchlight, we used a 3-voxel radius sphere. This yielded a map of classification accuracy for word1 and word2 centered at each voxel. Third, we binarized each dog's accuracy map with a threshold of .60 to indicate informative voxels. Finally, we spatially transformed each binarized accuracy map into template space and computed the average across dogs. This yielded the percentage of dogs with informative voxels at a given location. This map was thresholded at 0.2, showing only locations in which multiple dogs had informative voxels.

Following the general procedure of Stelzer et al. [41], we used random permutations of the attributes files to compute a null distribution of cluster sizes. For each dog, we computed 100 permutations of the attributes and the corresponding binarized searchlight map as above. We then randomly picked one of these 100 maps from each dog to compute a group accuracy map, which was done 1000 times, yielding 1000 null group maps. Using AFNI's 3dclust function, we then determined the clusters in each map (with options –NN1 and -1thresh 0.2 as above). We aggregated all 1000 cluster counts to create a cumulative distribution function for cluster size and count, which served as the null distribution.

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Results

Scan Day Discrimination Tests

Scans were scheduled as close as possible to the day on which object identification criterion was met (M = 9.33 days, SD =4.92 days) based on owner availability. On the day of the scheduled MRI scan, each dog was tested on her ability to behaviorally differentiate between the two trained objects out of 5 trials each. With the exception of Eddie, each dog correctly selected object 1 on 80 to 100 percent of the trials [M=85.73%, SE =3.87%], and object 2 on 60 to 100 percent of the trials [M=64.27%, SE=5.91%] (Fig. 4). The percent correct performance (subtracting 50 percent for chance levels of responding) on scan days for each object was compared in a mixed-effect linear model and showed that performance was significantly greater than chance [$F(1,11) = 35.85$, $P < 0.01$] and that there was a significant difference in performance between word1 and word2 [$F(1,11) = 21.8$, $P < 0.01$].

Parietotemporal ROI

Activation to the pseudowords was significantly greater than activation to the trained words as a main effect in both left and right parietotemporal regions [F (1, 11) = 5.01, P = 0.047]. Individual dogs displayed variable asymmetry in the left/right activation (Fig. 5), and although there was greater variability on the left side, on average, there was no significant difference between hemispheres [F (1, 11) = 2.07, P = 0.18]. There was no significant difference between the individual trained words [word1 - word2] [F (1, 11) = 1.34, P = 0.27], nor was there a significant difference between hemispheres [F (1, 11) = 0.07, P = 0.78]. There was no significant effect of ln(trial number) during the spoken words [F (1, 1098.14) = 0.64, P = 0.42] nor a significant interaction between ln(trial number) and [words - pseudowords] [F (1, 1095.21) = 1.25, P = 0.26]. Contrasts involving the presentation of objects were not examined in this ROI because they involved visual discrimination.

Caudate Nucleus ROI

There was no significant difference between the trained words and pseudowords in the caudate nuclei [F (1, 11) = 0.02, P = 0.89] nor was there a significant difference between hemispheres [F (1, 11) = .47, P = 0.5]. There was no significant difference between the trained words in the caudate nuclei [word1 – word2] [F (1, 11) = 0.34, P = 0.58], nor was there a significant difference between hemispheres [F (1, 11) = 0.99, P = 0.34].

Like the words, there was no significant difference between the trained objects in the caudate nuclei [object1 - object2] [F (1, 11) = 0.11, P = 0.74], nor was there a significant difference between left and right hemispheres [F (1, 11) = 0.1, P = 0.9]. Additionally, there was no difference between novel objects when presented in incongruent trails after the trained words, or when presented after pseudowords [incongruent – novel] [F (1, 11) = 0.18, P = 0.68], nor was there an effect of hemisphere [F (1, 11) = 0.0, P = 0.97].

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MVPA

Based on the null CDF of the permutated attribute maps at the chosen thresholds (>0.6 for informative voxels at the individual level; and 20% of subjects at the group level), the critical cluster size corresponding to *P*=0.05 was 326 voxels. The whole-brain searchlight of word1 vs. word2 revealed three clusters greater than this (Fig. 6): posterior thalamus/brainstem (2373 voxels, *P*=0.017); left temporoparietal region (886 voxels, P=0.028); and left dorsal caudate nucleus (372 voxels, *P*=0.045).

Discussion

In contrast to prior human studies, we found evidence for different neural mechanisms of both lexical and semantic processing in dogs. In human language studies, lexical processing typically shows greater cortical activation to words than to pseudowords. Our canine subjects demonstrated significantly greater BOLD response in the parietotemporal cortex to pseudowords than to trained words, which indicates differentiation between words and pseudowords. Thus, this provides evidence for lexical processing in dogs; however, familiarity with the trained words versus the novelty of pseudowords is the most likely explanation for differential activation. We argue that, at least in the scanner environment, auditory novelty, and not human-like language processing, may be the primary driver of dogs' response to words and pseudowords. Semantic processing – to the extent that we observed it with MVPA – was localized to a left temporoparietal region and caudate, suggesting an action-based processing of words rather than a purely symbolic one.

With regard to lexical processing, greater BOLD response to pseudowords versus real words is sometimes found in humans, especially in the bilateral superior temporal gyri [15,42], an area anatomically analogous to the parietotemporal region of interest of dogs in the present study. In humans, stronger activation to pseudowords depends on whether the pseudoword strongly resembles a known word or is so unlike known words as to prevent any semantic retrieval. If the pseudoword is similar to a known word, more processing is observed in the superior temporal gyri to recall the semantic content [15]. Like humans, dogs showed a similar trend in activation in the parietotemporal region potentially due to processing the difference between pseudowords and words. Thus, the greater activation to the pseudowords could be due to the perceived similarity between pseudowords and words that the dogs "knew." This would suggest that dogs can store semantic representations for nouns, and that they might generalize the meaning of a known word to words that sound similar. Though this is a possible alternate explanation, research on how dogs respond to altered phonetic characteristics of a command shows that they behaviorally discriminate between altered phonemes of well-known commands [9], suggesting that it is unlikely that the dogs in our study were confused by perceived similarity of words and pseudowords.

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The manner in which dogs learn words is different than humans do, and this undoubtedly affects their performance on behavioral tests and the patterns of brain activation we observed. Humans acquire nouns as early as 6 months of age and differentiate between nouns prior to their ability to use verbs [43,44]. In contrast, dogs do not typically have much experience with nouns because humans tend to train them on actions/verbs (e.g. sit and fetch). Consequently, even the trained words in our study were novel for the dogs in comparison to years' of experience with verbs as commands. Prior studies have shown only 3 dogs that consistently retrieved objects given a verbal referent [2,3]. Additionally, the same dogs had been trained to retrieve from a young age (<11 months), and in most cases rarely attained 100 percent accuracy. Object retrieval training for the current experiment was modeled from these studies; however, because the dogs' owners conducted training at home on a voluntary basis, training rigor could not be enforced. Another potential confound in our study was testing at the MRI center or the training facility, as opposed to the dogs' homes where training occurred. Thus a dog's behavior based on a command may vary based on the context in which it is given [6]. In addition, although human fMRI language studies do not typically repeat the spoken word each trial, it was necessary for the dogs to make sure that they heard each word and that enough fMRI volumes were obtained. Lastly, dogs might have habituated to the continued presentation of trained words followed by trained objects, as opposed to the single trial presentations of pseudowords and the accompanying novel objects. However, statistical tests showed that differential habituation of words relative to pseudowords did not occur within the parietotemporal ROI, making this an unlikely explanation for differential activation.

Instead, the dogs' greater activation to pseudowords compared to trained words supports a bias for novelty, as a dog's behavioral bias for novelty is often described as an explanation for performance otherwise labeled as learning by exclusion [23]. For example, a dog may select the novel item because it is novel among other stimuli, but not because it has learned all other stimuli and associates a new word with the novel item. Further, a dog's inherent preference for novelty could also explain performance initially labeled as learning by exclusion in previous behavioral studies [20,23,45]. A bias for novelty would therefore be reflected in the dog's brain as with her behavior. In our study, the salient aspect became whether or not the dog had heard the word before, or had seen the object before, rather than the dog forming a symbolic representation of the object.

Even so, we did not observe any difference in overall activation to word1 and word2, either in the parietotemporal ROI or caudate. Admittedly, the arbitrary labels attached to the trained words could explain the nonsignificant result in the univariate analysis. This raises the question of how the dogs were able to discriminate between the objects when given the spoken words outside the scanner. While some dogs showed a behavioral preference for one object over the other, this preference was not reflected in the caudate BOLD response to either trained word or trained object in the GLM contrast. So it seems unlikely that they discriminated based on relative reward values associated with the words. Searchlight MVPA, however, revealed three potential

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regions in which the words might be encoded: posterior thalamus/brainstem, left temporoparietal cortex, and left caudate. The cluster in the posterior thalamus included the inferior colliculi and medial geniculate nuclei, which are the primary pathways for auditory information. It is unclear why these low-level regions would discriminate words except for possible acoustic differences in the words themselves. More likely are the roles of the left temporoparietal region and caudate, suggesting a linkage between auditory processing of words and the motor/action system of the basal ganglia. If true, this suggests that dogs' comprehension of human language may not be symbolic per se, but rather associative to the actions taught during the training procedure. The lack of widespread cortical representations of words as in humans [10] supports the idea that a dog's representation of words is quite different than how humans represent language. Of course, these interpretations must be tempered by the small sample size compared to human studies, and even within this clusters, at best only half of the dogs had informative voxels.

So what do words actually mean to dogs? The imaging findings suggest an intimate linkage to action rather than symbolic representation. This is consistent with the idea that in the command to "go get [object]," the dog relies on the "go get" component, rather than the "[object]" portion [45]. In our study, dogs showed little evidence of differentiating between familiar words based on the overall BOLD response, and were given consistent retrieval commands with only the object's name changing. Although our adult dogs showed the ability to discriminate between two objects after months of training, none had previously been trained on this type of task. This difference in experience to nouns versus verbs during the developmental periods of humans and dogs might explain some of the disparity in dogs' linguistic processing ability. While dogs are frequently taught verbal commands for actions, they are less frequently taught the names of things. Our results may therefore be reflective of the dogs' developmental experiences as much as the architecture of their brains. Future fMRI research might even be used to improve communication between humans and dogs by way of measuring what dogs understand and how they learn it.

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Author Contributions

A.P., P.C., and G.B. designed and performed research; A.P, R.C., and G.B. analyzed data; A.P., P.C., G.B., & M. S. trained dogs; and A.P., G.B., P.C., R. C., and M.S. wrote the paper.

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FIGURES

Fig. 1. Individual dogs and their trained objects. All 12 dogs successfully trained to retrieve two objects using object names as verbal referents.

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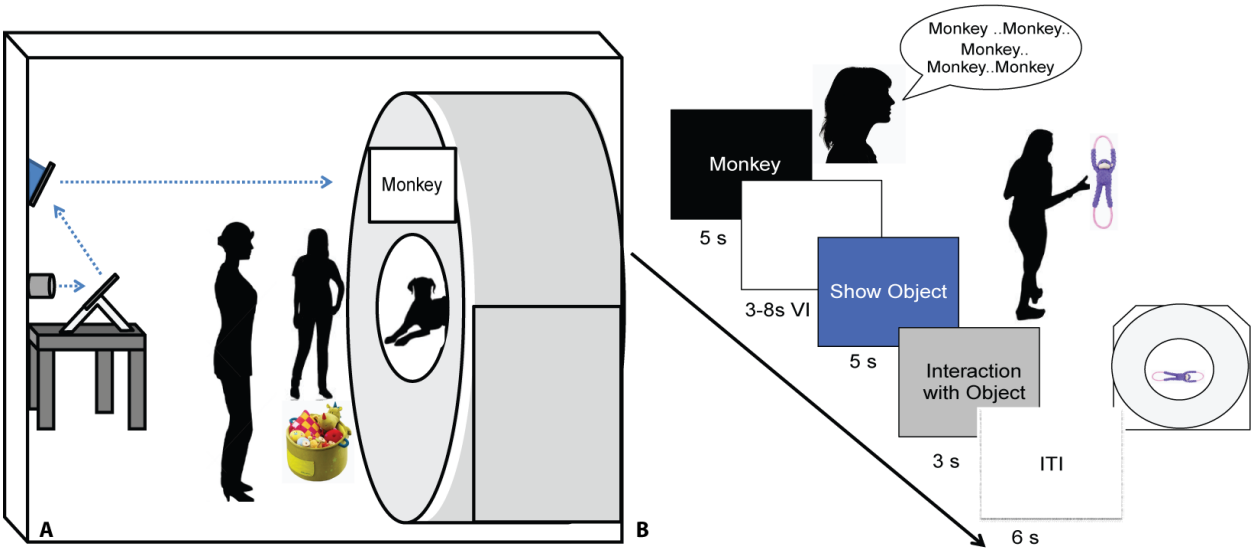

Fig. 2. Experimental design. A) Experimental setup with mirror relay projected words onto MRI surface. Owner is facing the projected word and her dog while the experimenter controls the presentation of words and objects to the owner. **B)** Trial timeline indicating spoken word over 5 s, 3-8 s VI, 5 s presentation of object, 3 s for the dog to interact with the object, followed by a 6 s ITI.

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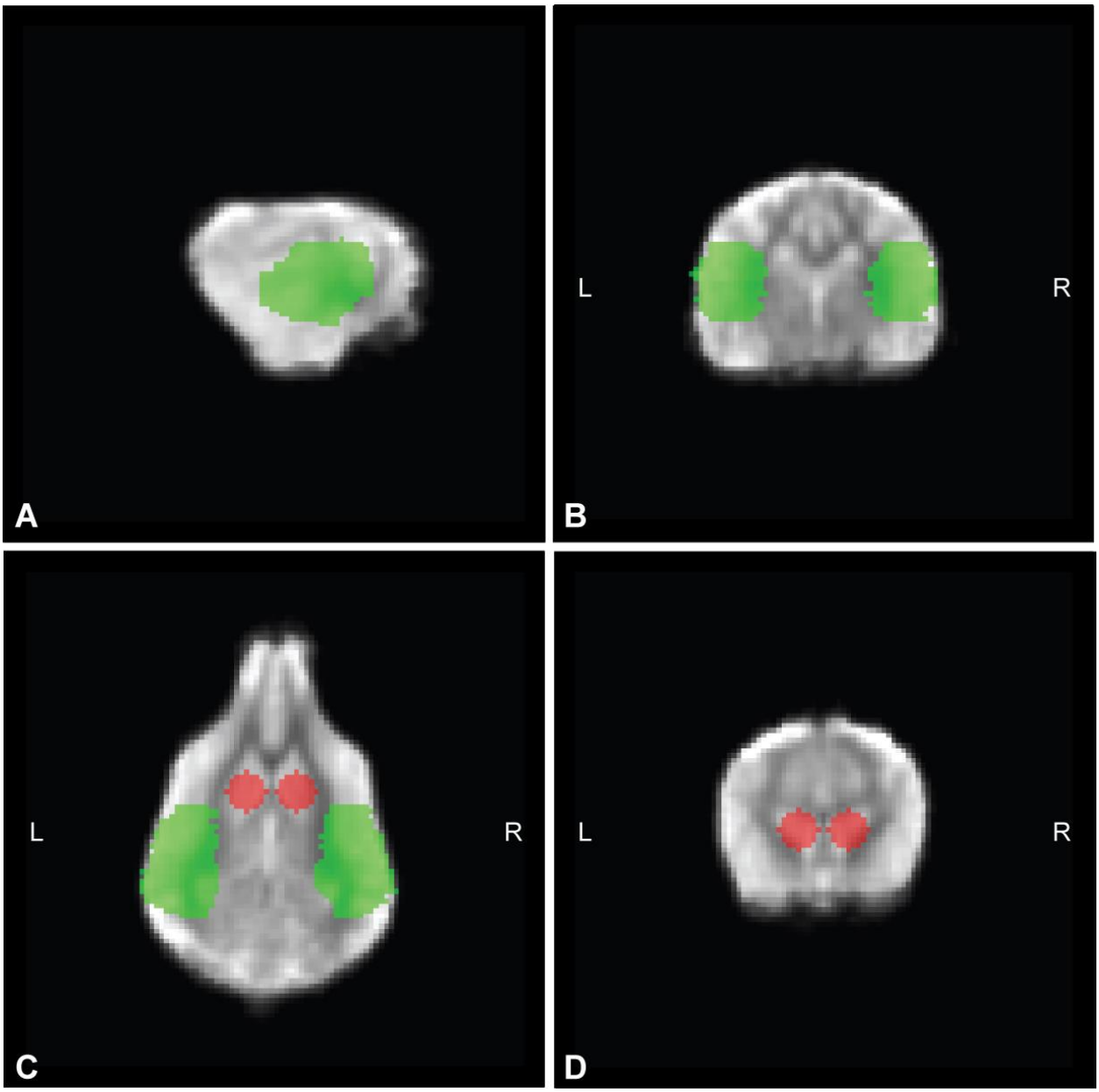

Fig. 3. Anatomical ROIs. Parietotemporal ROI (*green*) and caudate nucleus ROI (*red*) overlaid on mean structural image of all subjects after spatial transformation to template. **A)** Sagittal view of left hemisphere. **B)** Coronal image through temporal lobes. **C)** Transverse image. **D)** Coronal image through caudate.

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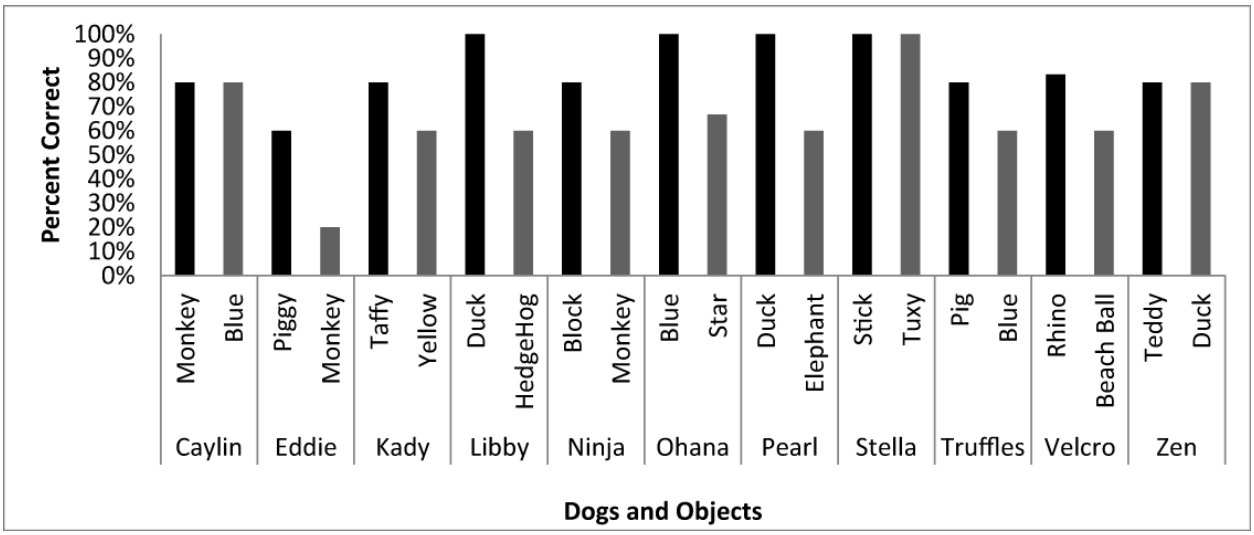

Fig. 4. Individual performance on two object discrimination tests. Tests were conducted on the day of the fMRI scan. Object 1 is in black, object 2 is in grey.

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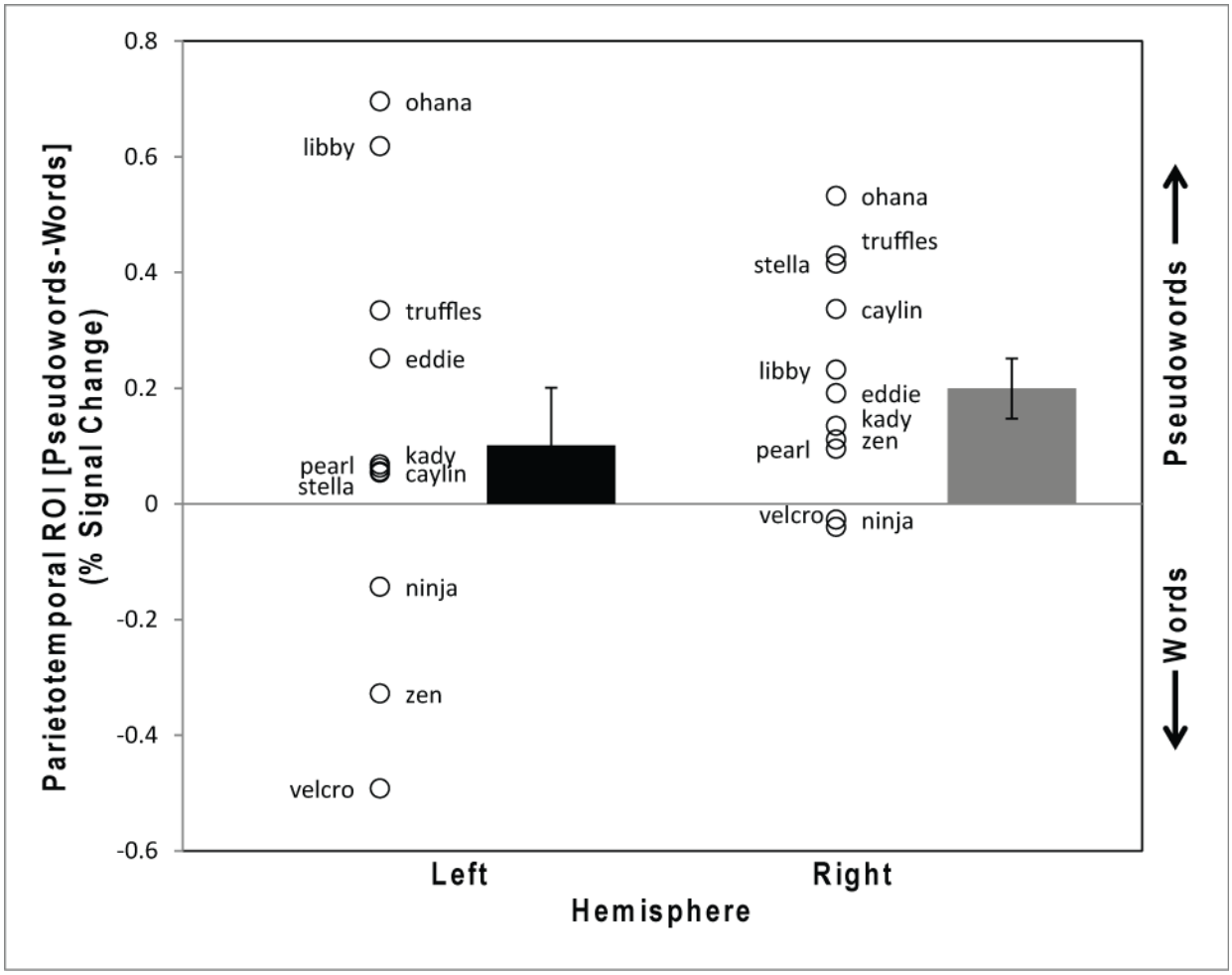

Fig. 5. Response in parietotemporal cortex to pseudowords and words. Individual beta values extracted from left and right parietotemporal mask including auditory cortex. Activation to the pseudowords was significantly greater than activation to the trained words as a main effect in both left and right parietotemporal regions [M = 0.16, SE = 0.07, F (1, 11) = 5.01, P = .047]. Bars represent the mean; error bars represent the standard error. Although individual dogs displayed variable asymmetry in the left/right activation, on average there was no significant difference between hemispheres [F (1, 11) = 2.07, P= 0.18].

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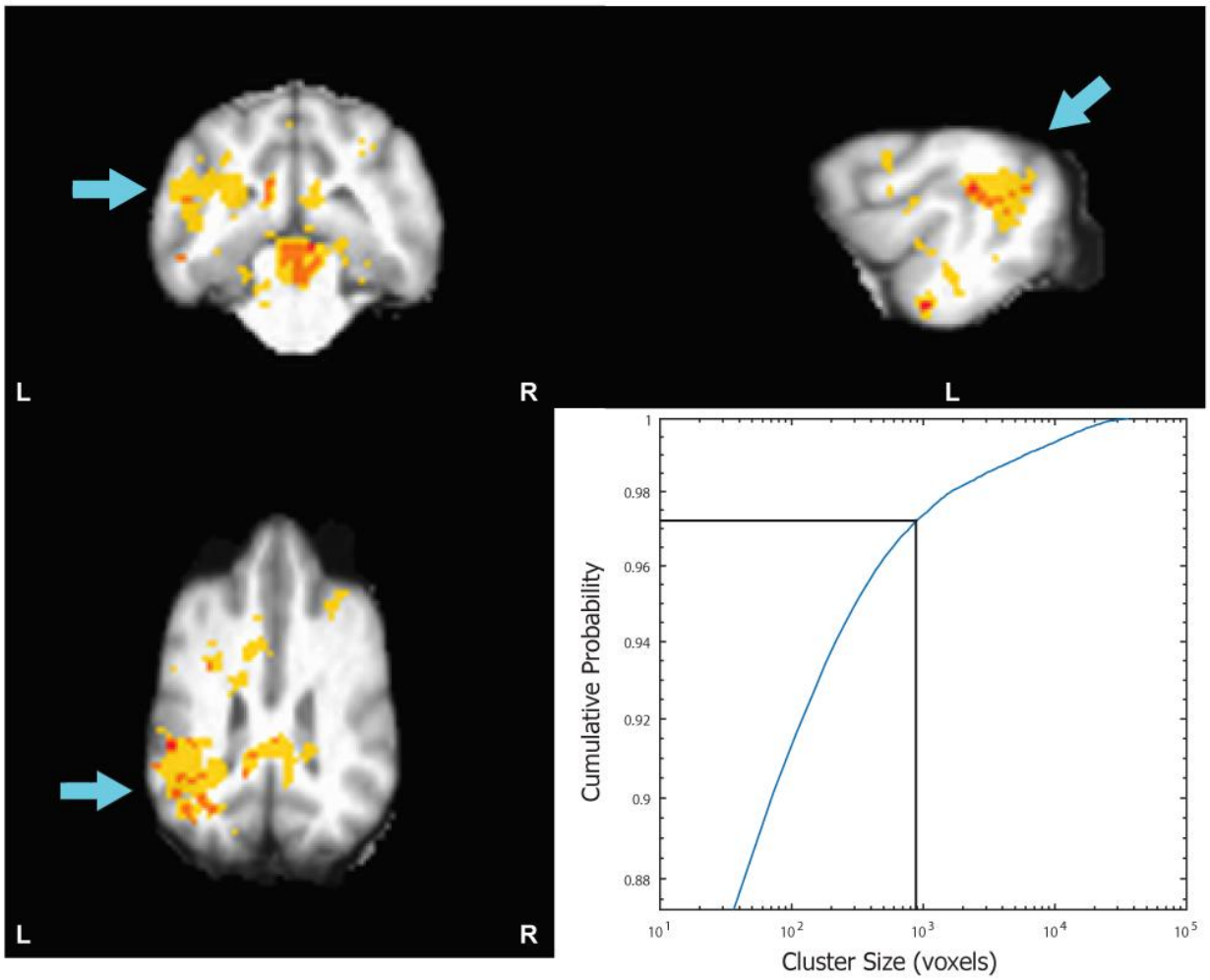

Fig. 6. Aggregate performance of searchlight MVPA classifier for word1 and word2 across dogs. Color intensity indicates percentage of dogs with informative voxels centered at each location. The image is thresholded such that only voxels with >20% of dogs are shown (*yellow*). The maximum value is 50% of dogs (*red voxels*). The arrow indicates a cluster in the left temporoparietal cortex whose cluster size was significantly above chance of the null distribution (P=0.028, *lower right*).