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Investigation of the Neural Mechanisms Underlying Perception in Dogs through Awake fMRI

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Doctor of Philosophy

Psychology: Neuroscience and Animal Behavior

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M.A., University of North Carolina Wilmington, 2014
B.S., Christopher Newport University, 2011

Advisor: Gregory Berns, Ph.D./ M.D.

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ABSTRACT

Although there has been a resurgence in the field of canine cognition, little research has focused on the neural mechanisms that underlie perception in domestic dogs. In this dissertation, I argue that functional magnetic resonance imaging (fMRI) provides a key insight into the role of brain regions that support stimulus-reward associations and brain-based biases for specific stimulus modalities in domestic dogs. The question is how these neurobiological mechanisms drive perceptual learning and discrimination in dogs, and how this varies based on the stimulus modality. This question was approached in three manuscripts using awake fMRI of 19 pet dogs. The first manuscript assessed whether reward systems in the dog brain process the predictive value of salient stimuli regardless of their sensory modality, or if some sensory modalities are privileged over others. The neurobiological learning curves of BOLD activation over time were compared to assess whether the rates of stimulus-reward acquisition were modality-dependent. We found that stimulus-reward associations were formed at a faster rate for olfactory and visual stimuli over verbal stimuli in reward processing regions of the dog brain. The second manuscript investigated the neural mechanisms of word discrimination in dogs using an auditory oddball paradigm consisting of trained words versus novel pseudowords. Dogs had greater neural activation to novel pseudowords relative to trained words in auditory processing regions. Multivoxel pattern analysis (MVPA) further revealed that a subset of dogs had clusters of informative voxels that discriminated between the two trained words in regions analogous to language processing regions in humans. The third manuscript identified brain regions important for processing object stimuli. We asked whether dogs' perception of objects is affected by a) the possible affordances dogs' associate with an object and b) whether the object is presented as a 2D or 3D version. We found that dogs have neural biases for processing object stimuli that the dog can interact with using their mouth and for the stimulus dimension with which they are most familiar. Together, these studies inform our current understanding of how dogs perceive their environment.

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GENERAL INTRODUCTION

How do dogs perceive their world? Although not much is known about dogs' internal experience, the study of how they perceive the world offers insight into what it might be like to be a dog. Understanding differences in humans' and dogs' perception facilitates our ability to empathize with dogs, which is important given that they are a domesticated species selectively bred to be our companions. Achieving this level of greater understanding will allow people to alter their behavior to better interact with, and in some instances to protect, dogs. In our endeavors to understand canine cognition we employ scientific methods to understand how dogs perceive the world, offering important comparisons to the human experience to bridge this perceptual gap.

The study of dogs' perception is especially interesting to dog owners, who coexist with dogs in the same home environment. In the United States alone, over 42.5 million households own one or more dogs[2]. In the modern-day home, humans invest substantial time and resources into dog ownership. For example, we spend about 12.4 billion dollars on veterinary services and 2.3 billion dollars on pet supplies annually in the U.S. [2, 3]. The pet industry offers technological innovations to maintain the human-dog bond when owners and their dogs are separated, including dog-cams and two-way video. Though this ability to interact digitally offers some peace of mind to owners when separated from their companions, we do not know what the dog gets out of digital interactions or how they perceive the information presented on a screen. What we do know is that dogs play an important role in humans lives and that we actively seek out opportunities to interact with our (or other's) dogs.

Rather than spend the day apart from their companions, dogs are increasingly making their way into workplaces, hospitals, and schools. Dogs typically enter these facilities as service animals or as emotional support animals, though some accompany their owners to workplaces that have open "Take Your Dog to Work" policies. The presence of dogs within the home and the classroom have numerous positive outcomes for children, including increased attention, motivation, mood, socio-emotional development, and decreased reports of loneliness and physiological responses to stress [4-6]. Parents of children with atypical development such as autism also report that having dogs in the home increases child safety, outdoor access, communication, and future social interactions[7-9]. In other locations, dogs also help to alleviate stress or anxiety reported by patients in hospitals as well as in the workplace and serve as a means for increased social interactions between people [10-13]. Collectively, dogs' presence at home and elsewhere is associated with positive experiences for the humans involved, and presumably, for the dogs as well.

Unfortunately, there are also instances where dog-human conflicts arise. Studies have shown that children are at an increased risk of severe injury such as facial bites because they do not interpret a dog's body and facial signals the same as adults, subsequently leading to the child's hospitalization and the rehoming or euthanasia of the dog [14, 15]. Humans who do correctly identify dogs' social cues do so because of experience and training, rather than dog ownership [15, 16]. Given dogs' many benefits to humans and their increasing proximity to humans, a growing body of research is needed to examine the mechanisms that facilitate interactions between human and dogs, as well as the social signals that, when misinterpreted, lead to negative outcomes for both the human and the dog. Insight into how dogs perceive the world can inform one side of these interactions, revealing species-specific differences in perception and potentially minimizing future dog-human conflicts by informing humans how to best interact with dogs. These differences likely occur because humans and dogs attend to different stimuli within their shared environment, such that the nature of the humans' experience may not be comparable to the dogs' experience in the same context. Studies on dogs' perception and how they interact with their environment can therefore offer insight into key features of the human-dog bond.

Despite dogs' proximity to and often total dependence on humans, *we do not know how dogs perceive the world*. The study of perception often involves the identification of behavioral or neural mechanisms, or both. In humans, we study perception by asking for a verbal response about the nature of an experience, whereas in nonhumans, we determine the nature of an animal's perception through behavioral measures. The difficulty, in addition to the absence of language, is in researchers figuring out how to ask the nonhuman the question. Using behavioral tasks, humans can infer things about the organism based on their responses, including how an organism interacts with its environment, which behaviors are likely to re-occur due to conditioning or reinforcement, and how animals are likely to interact with one another. But behavioral studies can only provide so much information about nonhumans, especially regarding mental representations or internal states.

The study of dogs' neural responses to stimuli offers a different approach to further understand perception. The discovery of neural mechanisms in nonhuman animals is principally useful because unlike human studies, verbal or written responses are not an option. Further, identification of neural mechanisms such as brain structures or circuitry that are conserved across species can indicate parallels between human and nonhumans in neural processes and cognition. As domestic dogs share the same environment with humans, they may fulfill a distinct niche in the investigation of conserved mechanisms underlying perception between humans and nonhumans.

Methods

Canine cognition research has relied on experimenter-reported measures of behavior as an indication of mental processes, which may not necessarily reflect the dog's experience. Part of the problem in studying dog cognition is the necessity of a behavioral response to demonstrate understanding. These studies rely on dogs performing the correct indication behavior, tracking the location of an object, or even using a touch screen in the presence of a handler [17-21]. For example, some dogs can retrieve a named object based on a command combined with the name of the object, but this often requires months of training [22-24]. This means that few other dogs have been documented to have this level of expertise, and that these dogs may be exceptional examples of the species [25-28].

The other problem is that studies of perception in nonhumans are difficult to interpret because behavioral measures are used to test whether an animal has formed a mental representation. Studies of dog cognition using visual stimuli have demonstrated behavioral evidence for the formation of relational concepts, object permanence, or object recognition [29-36]. But behavioral measures make it difficult to rule out alternate explanations for successful performance on tests, including simple mechanisms of discrimination like shape or color matching, novelty detection, or "Clever Hansing" [37]. It may be that most dogs rely on simple mechanisms of discrimination coupled with other cues from the human to figure out an appropriate behavioral response. Studies of dog behavior run the risk of overinterpretation, and because of this, it’s important to look for other sources of evidence, like adapting the methods of human studies to examine dogs’ perception.

Dogs have successfully adapted to technologies used in human research that are noninvasive and do not require restraint or sedation. These advances in research technologies offer the opportunity to directly retrieve information from the participant, and frequently do not require a human present, as in behavioral tasks. Dog studies have adapted to use eye-tracking, touch screens, electroencephalography (EEG), and functional magnetic resonance imaging (fMRI) [20, 38-40]. These measures offer similar control over experimental conditions as in human studies and afford the direct comparison between humans and dogs on similar tasks.

Neuroimaging is one method well-suited for identifying the neural mechanisms underlying a particular behavior. For example, fMRI has been used to study the valuation of stimuli based on the activation within brain structures like the striatum and that have been previously identified as measurable predictors of reward [41-43]. Comparison of the relative activation within these structures in humans has revealed neural biases for one type of reward over another (e.g. monetary, social, food) or one sensory modality over another (e.g. visual, auditory, odor) [44]. FMRI has also proved useful in the examination of neural mechanisms underlying different aspects of perception. Imaging studies of humans have identified a network of regions in the temporal and frontal cortices for language processing [45-48]. Imaging studies have also mapped the relevant structures in the olfactory pathway for the identification of and discrimination between odors such as the piriform, insula, amygdala and frontal gyrus [49-55]. Further, fMRI studies have begun to reevaluate commonly used visual stimuli within human studies of perception, noting that the object selective areas of the lateral occipital complex and posterior fusiform sulcus respond differently to real objects than to pictures of the objects, though it was previously assumed that the areas equate the two [56, 57]. This assumed equivalence between pictures and real-world stimuli is also a cautioned assumption in studies of dogs' perception [29, 58].

Awake fMRI of dogs may inform our knowledge about canine perception from the dog's perspective as previous dog fMRI research suggests that brain activation may yield significant insights into the dog mind [59]. Underlying neural mechanisms for processing sensory modalities may play a causal role in behavioral performances for working and service dogs. Like humans, fMRI can be used to elucidate the neural mechanisms underlying dogs' perception. Early dog fMRI studies demonstrated the replicability and reliability of caudate activation in response to hand signals predictive of food reward [40, 60, 61]. Later studies increased in complexity and duration paralleling human fMRI studies. For example, caudate and amygdala activation were correlated with specific aspects of a dog's temperament and could even be used as part of a biometric predictor for suitability as a service-dog [62]. And although initial studies relied on visual signals, later work suggested that both olfactory and verbal cues (e.g. social praise) could also elicit activity in the caudate [63, 64]. As in human imaging, functional localizers have also revealed areas of dogs' occipital cortex selective for processing human and dog faces [65-67]. Together these studies show that activation within areas of the dog brain can be used to predict perceptual or behavioral biases when processing of visual stimuli. To identify these mechanisms, the proposed studies using fMRI in dogs will directly compare neural activation during the presentation of multiple forms of stimuli to examine the neural mechanisms underlying modality-specific perception in the canine brain.

Current Studies

Here, we performed a series of fMRI experiments to address different aspects of perception in dogs. In the first manuscript, we used fMRI to measure the neural rates of associative learning in dogs to three modalities: visual, olfactory, and verbal. In three separate scanning sessions, each devoted to one modality, dogs were presented with two stimuli they had never encountered before. During each scan session, one of the stimuli (the conditioned stimulus) was always followed by a food reward, and the other (the control stimulus) nothing. If dogs formed modality-independent associations between the conditioned stimuli and reward, activity in the caudate nucleus should increase over time in response to the conditioned reward stimulus relative to the control stimulus, regardless of the modality. Similarly, if the amygdala functions as an attentional gate to learning, stimuli that are most salient to a dog (e.g. odorants) would result in greater activation in this structure. Lastly, if dogs preferentially process learning associations in one stimulus modality over another, then there will be a difference in the neural rate of learning between the three modalities.

In the second manuscript, to examine auditory processing in dogs, we used fMRI to measure activity in dogs' brains in response to both trained words and novel pseudowords. Over several months prior to scanning, owners trained their dogs to select two objects based on the objects' names. During the fMRI session, the owner spoke the names of the trained objects as well as novel pseudowords the dog had never heard before. If dogs discriminate target words from novel words as humans do, they should show differential activity in the parietal and temporal cortex in response to trained words relative to pseudowords [47, 68, 69]. In humans, this type of general semantic processing is associated with activity in a network comprised of the posterior inferior parietal lobe, middle temporal gyrus, fusiform and parahippocampal gyri, dorsomedial prefrontal cortex, and posterior cingulate gyrus [70]. In addition, if dogs use hedonic mechanisms to associate reward value with trained words, then differential activity should also be observed in the caudate.

In the third manuscript, we used awake fMRI of 16 dogs in three studies to examine the neural mechanisms underlying dogs' perception of dimensional objects. In Experiment 1, we performed a functional localizer for objects using the same movies that are used in human studies. Dogs participated in a second MRI scan encompassing a functional localizer to identify regions specific to object perception. The functional localizer included movies of novel faces, novel objects, the 3 trained objects, and scrambled images. If dogs form object representations based on shape, then activation in the dog brain to objects will be in areas homologous to the LOC. However, if object representations are grounded in affordances, then neural activation may extend for the affordance-associated objects into parietal regions of the "where" or dorsal stream. Like humans, neural activation to 2D version of object stimuli associated with affordances will be less than to the 3D objects because 2D stimuli do not have apparent affordances.

In Experiment 2, we used fMRI to test the effect of affordance on object representation. Prior to the MRI scan, we trained 12 dogs to grasp one object with their mouth and to touch one object with their paw. A third object was presented to the dog but not interacted with, to serve as a control. During the scan session, dogs were presented all three trained objects by their owners. Neural activation was compared during the presentations of all three objects within functionally defined regions associated with object perception and regions for planning motor actions, such as the anterior intraparietal sulcus (aIPS). If dogs discriminate between object stimuli based on their affordances (e.g. fits in mouth), then we would expect there to be differential neural activation in parietal regions between the two objects based on their trained affordances.

In Experiment 3, we used fMRI to measure activity in dogs' brains in response to both objects and pictures of the objects. Prior to scanning, 15 dogs were split into two groups. Dogs in the first group were trained on two 3D object stimuli, one of which was associated with reward and the other with nothing. Dogs in the second group were trained on two 2D picture stimuli, and like before, one was associated with reward and the other with nothing. During the fMRI session, dogs from both groups were presented both the picture stimuli and object stimuli. If dogs equate 2D and 3D stimuli, then they should show no difference in neural activity between the object and the picture in areas such as the LOC. Further, if hedonic mechanisms facilitate abstraction from 2D to 3D versions of object stimuli associated with reward, then dogs will show greater neural activity for the trained reward stimulus than the no reward stimulus, and similar trends in activation to the untrained stimuli.

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Manuscript 1

Fast Neural Learning in Dogs: A Multimodal Sensory fMRI Study

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ABSTRACT

Dogs may follow their nose, but they learn associations to many types of sensory stimuli. Are some modalities learned better than others? We used awake fMRI in 19 dogs over a series of three experiments to measure reward-related learning of visual, olfactory, and verbal stimuli. Neurobiological learning curves were generated for individual dogs by measuring activation over time within three regions of interest: the caudate nucleus, amygdala, and parietotemporal cortex. The learning curves showed that dogs formed stimulus-reward associations in as little as 22 trials. Consistent with neuroimaging studies of associative learning, the caudate showed a main effect for reward-related stimuli, but not a significant interaction with modality. However, there were significant differences in the time courses, suggesting that although multiple modalities are represented in the caudate, the rates of acquisition and habituation are modality-dependent and are potentially gated by their salience in the amygdala. Visual and olfactory modalities resulted in the fastest learning, while verbal stimuli were least effective, suggesting that verbal commands may be the least efficient way to train dogs.

INTRODUCTION

It is well known that dogs have keen sensory abilities, but are some modalities learned better than others? For example, a dog's behavior is popularly considered to be driven by their noses [1]. On the other hand, dogs have superior hearing than humans and readily form visual associations – even being able to discriminate human facial expressions [2-4]. The experimental literature has shown that dogs can learn associations to almost any stimulus, but demonstrating that certain modalities are innately preferred to others has been difficult [5]. Apart from the basic question of how different sensory modalities impact associative learning in dogs, the answer could affect how dogs are trained in an optimal manner.

While behavioral mechanisms underlying associative learning are well-described, there has been increasing sophistication in neural methods to understand how these associations are formed in the brain. In humans, functional magnetic resonance imaging (fMRI) has become the preferred neuroscience tool because of its noninvasiveness. Coupled with computational models, this approach has been successful in parsing the contributions of different brain structures to reinforcement learning. Several fMRI studies have demonstrated that the striatum "learns" the value of visual stimuli in a manner consistent with reward-prediction error models, regardless of whether the reward is a primary taste reward or money [6-10]. Similar results have been obtained for visual cues that predict pleasant and unpleasant odors, although the time courses varied by the nature of the odor and brain region (e.g. striatum, orbitofrontal cortex, or amygdala) [11]. More generally, the amygdala has been hypothesized to interact with the reward-learning process by gating attention to salient stimuli [12, 13].

Like humans, dogs can be trained for non-invasive fMRI studies [14]. Early dog-fMRI studies demonstrated the replicability and reliability of caudate activation in response to hand signals predictive of food reward [15]. Later studies extended these results and showed that caudate and amygdala activation were correlated with specific aspects of a dog's temperament and could even be used as part of a biometric predictor for suitability as a service-dog [16]. Although initial studies relied on visual signals, later work suggested that both olfactory and verbal cues (e.g. social praise) could also elicit activity in the caudate [17, 18].

Here, we used fMRI to measure the neural rates of associative learning in dogs to three modalities: visual, olfactory, and verbal. In three separate scanning sessions, each devoted to one modality, dogs were presented with two stimuli they had never encountered before. During each scan session, one of the stimuli (the conditioned stimulus) was always followed by a food reward, and the other (the control stimulus) nothing. If dogs formed modality-independent associations between the conditioned stimuli and reward, activity in the caudate nucleus should increase over time in response to the conditioned reward stimulus relative to the control stimulus, regardless of the modality. Similarly, if the amygdala functions as an attentional gate to learning, stimuli that are most salient to a dog (e.g. odorants) would result in greater activation in this structure. Lastly, if dogs preferentially process learning associations in one stimulus modality over another, then there will be a difference in the neural rate of learning between the three modalities.

MATERIALS AND METHODS

Participants

Participants were 19 pet dogs volunteered by their Atlanta owners for fMRI training and fMRI studies [14, 15, 18, 19]. All dogs had previously completed one or more scans for the project and had demonstrated the ability to participate in awake fMRI scans. The study utilized previously neutral stimuli and no physical or chemical restraint was implemented. 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 (Protocols DAR-2002879-091817BA and DAR-4000079-ENTPR-A), and all owners gave written consent for their dog's participation in the study.

Experimental Design

Dogs entered and stationed themselves in custom chin rests in the scanner bore. All scans took place in the presence of the dog's primary owner, who stood throughout the scan at the opening of the magnet bore, directly in front of the dogs, and delivered all rewards (hot dogs) to the dog. The owner was present to minimize any anxiety that the dog may experience due to separation, consistent with studies involving pets or human infants. An experimenter was stationed next to the owner, out of view of the dog. The experimenter controlled the timing and presentation of stimuli to the owners and the dogs via a four-button MRI-compatible button box. Onset of each stimulus was timestamped by the simultaneous press of the button box by the experimenter. Manual control of the stimuli by the experimenter was necessary, as opposed to a scripted presentation, because of the variable time it takes dogs to consume food rewards.

In three separate scanning sessions on different days, each devoted to one modality, dogs were presented with two stimuli they had never encountered before. In each session dogs were presented with either two objects, two odors, or two spoken words. All dogs completed the scan sessions in the same order (objects, odors, words), and all data collection for one modality was completed for all dogs prior to any data collection with the next modality. An event-based design was used, consisting of reward or no-reward trial types, where one stimulus within a modality was associated with the receipt of reward and the other stimulus with no-reward. On reward trials, the selected stimulus was presented for a fixed duration, which was followed by the delivery of a food reward. During no-reward trials, the second stimulus was presented for the same fixed duration and was followed by nothing. Trials were separated by an inter-trial interval specific to each modality as described below, and each dog received the same trial sequence.

Each scan session consisted of 4 runs, lasting approximately 9 minutes per run. Each run consisted of 22 trials (11 reward, 11 no-reward) with a semi-randomized presentation order, for a total of 88 trials per scan session. No trial type was repeated more than 4 times sequentially, as dogs could habituate to the stimulus, or may have a higher probability of exiting the scanner if a reward had not been issued recently. Following each run, dogs would exit the scanner and relax, drink water, or stay in the scanner to complete the next run.

Scanning was conducted with a Siemens 3 T Trio whole-body scanner using procedures described previously [14, 15]. During the first of the three scans sessions, 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. Four runs of up to 400 functional volumes were acquired for each subject, with each run lasting about 9 minutes.

Visual Stimuli

A plastic pineapple and an inflatable flamingo were used (Fig. 1A). Based on owner responses, no dog had experience with the objects prior to the scan. One object was presented at a time, held at the dog’s eye level directly at the opening of the bore for 8 s, followed by delivery of a reward (hot dog) or nothing. Trials were separated by a 7 s inter trial interval. Dogs were semi-randomly assigned the pineapple or the flamingo as the reward stimulus such that roughly half of the dogs were assigned to each group (see Table 1).

Olfactory Stimuli

Olfactory stimuli were aqueous solutions of isoamyl acetate (IA) and hexanol (Hex) calculated to result in approximately 5 ppm in the headspace of the container. Partial vapor pressures were calculated based on the molecular weight and reported vapor pressures of 4 mmHg and 0.9 mmHg respectively, obtained from PubChem (pubchem.ncbi.nlm.nih.gov). The odorants were miscible with water and the partial pressure of the odorant was the product of the pure odorant vapor pressure and the mole fraction of the odorant. The final dilutions in water were 0.15 mL/L for IA and 0.55 mL/L for Hex.

Odorants were delivered via a stream of air from an aquarium grade air pump (EcoPlus Commercial Air Pump 1030 GPH) through a Drierite filter (drierite.com), and afterwards through a 3-way plastic splitter to two plastic 100 mL jars containing 50 ml of odorant solutions and one jar containing 50 ml of water to serve as a control. Each solution mixed with a continuous air stream. Plastic valves were used to control directional flow of odorized air through 10’ of 1/8” ID Teflon tube, where the mixture (air dilution of the odorant) exited a PVC tube with a 1” diameter opening positioned in the MRI bore 12” from the dog’s snout (Fig. 1B). The third tube carrying air from the control jar remained open throughout the presentations of odorized air, maintaining a steady air stream presented to the dog and assisting in the clearing of lingering odor within the magnet bore. Dogs were presented an odor for an initial 3.6s during a span of 7.2 s, followed by a reward (hot dog) or nothing, with a 9.6 s inter trial interval between odor presentations. The inter trial interval was increased compared to the visual stimulus scans to ensure that the odorant within the magnet bore had cleared prior to the next trial. Dogs were semi-randomly assigned IA or Hex as the reward stimulus such that roughly half of the dogs were assigned to each group (see Table 1).

Verbal Stimuli

Verbal stimuli were the words “Callooh” and “Frabjous” from the Lewis Carroll poem, “Jabberwocky.” The words were chosen as novel pseudowords to the dog. The words were spoken by the dog’s primary owner, who was positioned in front of the dog at the opening of the magnet bore. Both owners and dogs wore ear plugs, reducing scanner noise by 30 decibels, but allowing for intelligible speech over the scanner noise. The words were intelligible to the experimenters, who also wore ear plugs while next to the MRI during scanning, as well as the human operators in the control room through the intercom. At the start of each trial, a word was presented to the owners via a mirror relay system that projected the words onto the surface of the scanner, directly over the owner's head (Fig 1C). Owners were positioned in front of the dog and repeated the words five times for an average duration of 6 s. Words were repeated to ensure the dogs heard them. Words associated with reward were followed by a 4 s delay, then the delivery of a food reward, and words not associated with a reward were followed by nothing. The words were followed by a delay after their presentation for three reasons. First, a previous imaging study by our lab where dogs were presented with spoken words by their owners in the MRI showed that dogs may move initially upon hearing words, likely due to their familiarity with verbal commands, resulting in loss of data for those instances. This was also the reasoning behind the repetition of the verbal stimuli. Second, the verbal stimuli could be delivered at a much faster rate than the presentation of the objects. The rate of the delivery of the odor stimuli through the olfactometer was also dependent on the manual operation of the olfactometer by the experimenter and the length of the tube carrying scented air from the olfactometer jar to the dog's nose. Third, if there was no delay, the timing of the BOLD response following the verbal stimuli would peak at the moment that the reward or nothing was delivered following the last repetition, resulting in additional loss of data due to movement. Trials were separated by an 8.4 s inter trial interval. Dogs were semi-randomly assigned "Frabjous" or "Callooh" as the reward stimulus such that roughly half of the dogs were assigned to each group (see Table 1). Dog Mauja was deaf, and so did not participate in the verbal stimuli experiment. Dog Libby had excessive motion in this experiment and was not included in the analysis for this stimulus modality.

Table 1. **Dogs (N=19) and stimuli paired with reward**.

Dog	Breed	Sex	Reward Object	Reward Odor	Reward Word
BhuBo	Boxer mix	M	Pineapple	hexanol	Callooh
Caylin	Border collie	F	Pineapple	hexanol	Frabjous
Daisy	Pitbull mix	F	Flamingo	hexanol	Callooh
Eddie	Labrador Golden mix	M	Pineapple	isoamyl acetate	Callooh
Kady	Labrador	F	Flamingo	hexanol	Frabjous
Koda	Pitbull mix	F	Flamingo	isoamyl acetate	Callooh
Libby	Pitbull mix	F	Pineapple	hexanol	Callooh
Mauja	Cattle dog mix	F	Pineapple	hexanol	N/A
Ninja	Cattle dog mix	F	Flamingo	isoamyl acetate	Frabjous
Ohana	Golden Retriever	F	Pineapple	hexanol	Frabjous
Ollie	Border collie Beagle mix	M	Flamingo	isoamyl acetate	Frabjous
Ozzie	Bichon-Yorkie mix	M	Flamingo	isoamyl acetate	Frabjous
Pearl	Golden Retriever	F	Pineapple	hexanol	Frabjous
Tallulah	Cattle Dog mix	F	Flamingo	hexanol	Callooh
Truffles	Pointer mix	F	Pineapple	isoamyl acetate	Frabjous
Tug	Portuguese Water dog	M	Flamingo	hexanol	Callooh
Velcro	Viszla	M	Pineapple	isoamyl acetate	Frabjous
Wil	Australian Shepherd	M	Pineapple	isoamyl acetate	Callooh
Zen	Labrador Golden mix	M	Flamingo	isoamyl acetate	Callooh

Dog's names, breed, sex, and stimuli (S+) are listed

Statistical Analyses

Preprocessing

Preprocessing of the fMRI data included motion correction, censoring, and normalization using AFNI (NIH) and its associated functions. Two-pass, six-parameter rigid-body motion correction was used based on a hand-selected reference volume for each dog that corresponded to their average position within the magnet bore across runs. Aggressive censoring removed unusable volumes from the fMRI time sequence because dogs can move between trials, when interacting with the object, smelling an odor, hearing a word, 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 in previous studies [18]. The Advanced Normalization Tools (ANTs) software was used to spatially normalize the mean of the motion-corrected functional images [20] to the individual dog's structural image.

General Linear Model

Each subject's motion-corrected, censored, smoothed images were analyzed within a general linear model (GLM) for each voxel in the brain using 3dDeconvolve (part of the AFNI suite). Motion time courses were generated through motion correction, and constant, linear, quadratic, cubic, and quartic drift terms were included as nuisance regressors. 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 for each experiment were modeled using AFNI's dmUBLOCK and stim_times_IM functions and were as follows: (1) reward stimulus; (2) no-reward stimulus. The function creates a column in the design matrix for each of the 88 trials, allowing for the estimation of beta values for each trial. The reason for this approach was that even though the motion censoring flagged problematic volumes, it is possible that spin-history effects could result in spurious levels of activation in specific regions of interest that, when averaged over an entire run, could still affect beta estimates. Trials with beta values greater than an absolute three percent signal change were removed prior to analyses (assuming that these were beyond the physiologic range of the BOLD signal). As described next, we used the trial-by-trial betas to estimate trimmed-means from the remaining beta values.

Region of Interest (ROI) Analysis

As our interest was based on the dog's changing response to novel visual, olfactory, or verbal stimuli, all quantitative analyses based on the imaging results used activation values in the canine brain area previously observed to be responsive to visual [18], olfactory [17, 21], and verbal [22] stimuli. Anatomical ROIs of the left and right caudate nuclei, and the left and right amygdala were defined structurally using each dog's T2-weighted structural image of the whole brain. A parietotemporal region was also included because of its known involvement with verbal and visual stimuli in dog fMRI studies but no reported involvement with stimulus valuation [22]. The parietotemporal region of interest was defined using a high-resolution canine brain atlas [23] and applyANTSTransformation (part of the ANTS suite) to transform the left and right parietotemporal ROIs from template to individual space (Fig 2). Thereafter, all analyses were performed in individual, rather than group space.

Beta values for each presentation of reward stimuli (44 trials) and no-reward stimuli (44 trials) were extracted from and averaged over the ROIs in the left and right hemispheres. Beta values were used to construct a learning curve across presentations of the stimuli by ROI, run, and modality, as well as to test for any hemispheric differences. We used the mixed-model procedure in SPSS 24 (IBM) with fixed-effects for the intercept, run number, type (reward or no-reward), modality (visual, olfactory, & verbal), ROI (amygdala, caudate, & parietotemporal), and hemisphere (left or right), identity covariance structure, and maximum-likelihood estimation. Run was modeled as a fixed effect because it made no assumptions about the time course. As hemisphere did not account for a significant amount of variance, all analyses removed hemisphere as a factor.

RESULTS

We found neural evidence for differentiation of the reward and no-reward stimuli in all modalities ($p < 0.001$) (Table 2). Although the amplitude of this difference varied by ROI ($p = 0.014$), there was only a marginally significant interaction with modality ($p = 0.045$). However, the modality significantly affected the temporal pattern of the difference between reward and no-reward stimuli across Run ($p = 0.006$).

Table 2. Model results for Reward vs. No Reward, Run, Modality, and ROI.

Fixed Effects	**Numerator df**	**Denominator df**	***F***	***Sig.***
Intercept	1	17.757	11.105	0.004
Run	3	23706.431	4.801	0.002
Rew_NoRew	1	23696.466	35.034	0.000
Modality	2	23192.742	10.798	0.000
ROI	2	23704.765	33.667	0.000
Run * Rew_NoRew	3	23692.763	3.359	0.018
Run * Modality	6	23703.114	2.794	0.010
Run * ROI	6	23690.984	2.072	0.053
Rew_NoRew * Modality	2	23695.363	3.102	0.045
Rew_NoRew * ROI	2	23690.649	4.284	0.014
Modality * ROI	4	23701.672	5.389	0.000
Run * Rew_NoRew * Modality	6	23693.671	3.039	0.006
Run * Rew_NoRew * ROI	6	23690.457	0.423	0.864
Run * Modality * ROI	12	23691.077	0.827	0.623
Rew_NoRew * Modality * ROI	4	23690.700	0.537	0.709
Run * Rew_NoRew * Modality * ROI	12	23690.415	0.461	0.938

As there was differentiation of the reward and no-reward stimuli in all modalities, we used post-hoc analyses to examine whether these differences remained when segregated by

ROI and a Bonferroni correction for multiple comparisons. In the caudate (Fig. 3A), there was a significant main effect of [Reward – No Reward] ($p = 0.013$) but not the interaction with modality ($p = 0.081$), consistent with general reward processing. There was no interaction with [Reward – No Reward] and Run, but the interaction of [Reward – No Reward] x Run x Modality was significant ($p = 0.018$), indicating that the time course of the differentiation of value varied by modality. For the caudate, both the visual and olfactory stimuli showed a rising differentiation by Runs 2 & 3, with some decrement by run 4 for olfaction.

A similar, more pronounced, pattern was observed in the amygdala (Fig. 3B). Like the caudate, the amygdala displayed a significant main effect of [Reward – No Reward] ($p < 0.001$) but no interaction with modality ($p = 0.238$). There was not a significant interaction of Run x [Reward – No Reward] ($p = 0.584$), indicating that the amygdala "learned" the differential values of the stimuli in Run 1 and maintained them throughout each experiment. Unlike the caudate, there was not a significant interaction of [Reward – No Reward] x Run x Modality ($p = 0.707$), indicating that the modality did not affect the rate of learning or habituation.

Finally, the parietotemporal cortex (Fig. 3C) also showed a main effect for [Reward – No Reward] ($p = 0.021$), but this was of marginal significance and would not survive Bonferroni correction for three separate analyses.

In sum, the neural learning curves showed that dogs formed stimulus-reward associations in as little as 22 trials. However, there were significant differences in the time courses, suggesting that the rates of acquisition and habituation were modality-dependent, with visual and olfactory modalities resulting in the fastest learning (Fig. 3D), while verbal stimuli were least effective.

DISCUSSION

In three experiments, we demonstrated the use of fMRI in dogs to compare associative reward-learning in the brain across visual, olfactory, and verbal modalities. Consistent with reward learning in neuroimaging studies, the caudate showed main effects for reward-related stimuli but not a significant interaction with modality. However, there were significant differences in the time courses, suggesting that although multiple modalities are represented in these structures, the rates of acquisition and habituation are modality-dependent. Further, we demonstrate that dogs have neural mechanisms that support a bias for learning conditioned visual and olfactory stimuli more rapidly than verbal stimuli.

While many fMRI studies have shown that the striatum differentially responds to conditioned stimuli associated with reward, this is the first fMRI study that directly compares reward learning across three modalities in the same participants. The significant differential effect for reward versus no-reward across multiple ROIs suggests that reward regions of the canine brain such as the striatum process the value of conditioned stimuli regardless of modality. Post-hoc analyses revealed that the primary structures associated with the differentiation of value between conditioned stimuli were the caudate and amygdala, not the parietotemporal region. Moreover, the differentiation of value was more pronounced for visual and olfactory stimuli. Interestingly, the parietotemporal cortex, which was originally selected because of its known involvement with visual and auditory stimuli, turned out to have the strongest effect for visual and olfactory stimuli. This multimodal activation can be attributed to inclusion of both primary auditory and parietal cortices within the defined region.

Although it is debatable whether the amygdala should be considered part of the "reward" circuit, its role in associative learning is well-established. One recent model suggests that the amygdala computes the surprisingness of stimuli while the striatum computes reward prediction errors [12]. A hallmark of this model is that surprise declines with repeated exposure while prediction errors remain constant as long as the stimuli themselves are unpredictable. This is exactly the pattern we observed in the caudate and the amygdala (Fig. 3), which appeared largely independent of modality. Within this framework, the amygdala activation can be interpreted as an attentional "gate" that signals the salience of a stimulus, setting up the reward system to compute its value. Further insight is gained by examining the time courses of activation in these regions.

Our results show that dogs acquired the reward associations with odors and visual stimuli at a different time course than verbal stimuli. The neural activation for visual and olfactory stimuli within the caudate and amygdala peaked by the second run, indicating the conditioned associations were formed within 22 trials. This is inconsistent with dog behavioral studies, which require days to form the stimulus-reward associations of visual or odor stimuli to reach a behavioral criterion [24-26]. However, our findings are consistent with human fMRI studies, where word learning was reported to occur at a slower rate during associative learning than visual learning [27].

The effects of stimulus modality on differential neural time courses highlight the potential implications for training dogs. Most training protocols for dogs use gestural and verbal commands. While optimal for humans, these protocols may not be the most effective for learning from a dog's perspective. Our results are consistent with previous behavioral findings that suggest dogs prioritize gestures over verbal commands when presented with conflicting signals [5, 28]. Effective processing of visual information is essential to the social success and safety of the dog. Dogs frequently use body language as a principal mode of dog-dog communication. Tail wagging, facial expressions, and body postures are obvious examples[29-34]. In addition to visual cues, dogs use odors as a means for gaining social information from both humans and dogs[35, 36]. When olfactory information is present and relevant, the dog may consider olfactory sensory information prepotent over visual information [37]. Although dogs may attend to verbal stimuli, olfactory and visual stimuli likely have greater importance in the dog's assessment of its physical and social environment and when interacting within such environments. Our results, showing greater salience for olfactory and visual stimuli in the amygdala, are concordant with the dogs' behavioral preferences in their natural surroundings.

There are several limitations to our study. First, although we isolated the salient modality in three separate experiments, the presence of the human owner was constant. Because the human was not blind to the nature of the stimuli, they could have inadvertently influenced the associative process through body language. However, because the olfactory modality was the most effective in eliciting reward-associations across all ROIs, and the olfactory stimuli were least likely to be picked up by the humans and were not saliently communicated by human owners, as were the display of the visual objects or the vocalization of the auditory stimulus, so-called 'Clever Hans' effects are unlikely to explain these results. Second, although the verbal stimuli were the least effective in forming reward-associations, this may have more to do with the discriminability of words in the scanner environment. Although the words were distinguishable to the experimenters over the scanner noise, it may have been more difficult for the dogs. There is some evidence that dogs can discriminate between spoken words during an fMRI scan, as a previous study where owners spoke trained words and pseudowords to their dogs during scanning showed neurobiological evidence that dogs were differentiating between the words in primary auditory cortices and the parietotemporal cortex[38]. This and previous results suggest some mechanistic similarity between humans and dogs for the rate of associative learning of verbal stimuli relative to other modalities. Third, we found only a marginally significant interaction between reward and modality ($p = 0.045$). Given the large sample size and high number of observations, we conclude that this is probably not a significant effect, especially since the other effects had markedly smaller *p*-values. Even so, a non-significant result does not mean that the effect doesn't exist. It is possible that the modality of the conditioned stimuli affected the magnitude of the representation in reward-related structures like the caudate and amygdala. Undoubtedly the differential value of stimuli would be influenced by their discriminability, and as already noted, verbal cues were at a disadvantage. Fourth, the effects of habituation counteract those of learning. Habituation was perhaps most evident in the amygdala, which displayed a generally declining response with run, regardless of the modality. There is ample evidence that the amygdala habituates to repeated presentations of the same stimuli [39-41]. It would not be surprising that repeated presentation of the stimuli could lead to decreased physiological response, especially to odors. Most dogs included in the study also had experience from previous fMRI studies with conditioned object-reward associations, and some with conditioned word-object associations, such that odors within the scanner environment may have been more novel than other stimulus modalities. Finally, the stimulus-reward associations were acquired through a passive task in the scanner.

No behavioral tests were conducted to test acquisition of the learned associations or to compare to the neural activations.

In summary, our results show that associative learning may be measured across multiple modalities in the caudate and that stimulus salience is denoted by the amygdala. However, certain modalities – notably visual and olfactory – were more effective in eliciting reward-related responses, especially in the rate at which they were acquired. Our results suggest that the human inclination for verbal communication appears to be based on human preferences, rather than the dog's innate aptitude. Consequently, pet and working dog training programs would likely become more productive, with accelerated learning rates for the dog, if commands were introduced via hand signals or other physical modes of communication.

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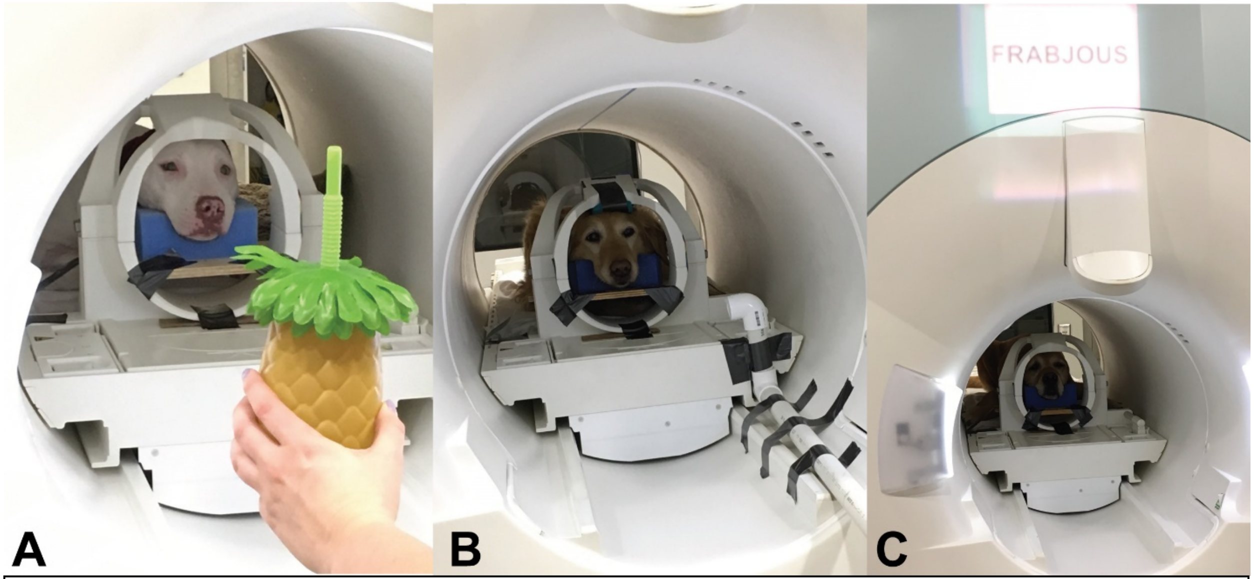

Figure 1. Experimental design with conditioned stimuli. Two novel stimuli were repeatedly presented during three scanning sessions, each devoted to one stimulus modality. One stimulus was associated with food (Reward), one associated with nothing (No Reward). **A.)** Presentation of pineapple object by owner to dog in MRI bore during visual modality session. **B.)** Presentation of odorants to dog in MRI bore via experimenter-controlled olfactometer during olfactory modality session. The owner remained in front of the dog. **C).** Presentation of pseudoword *Frabjous* to owner projected above MRI bore opening during verbal modality session. The owner spoke the projected word five times per trial.

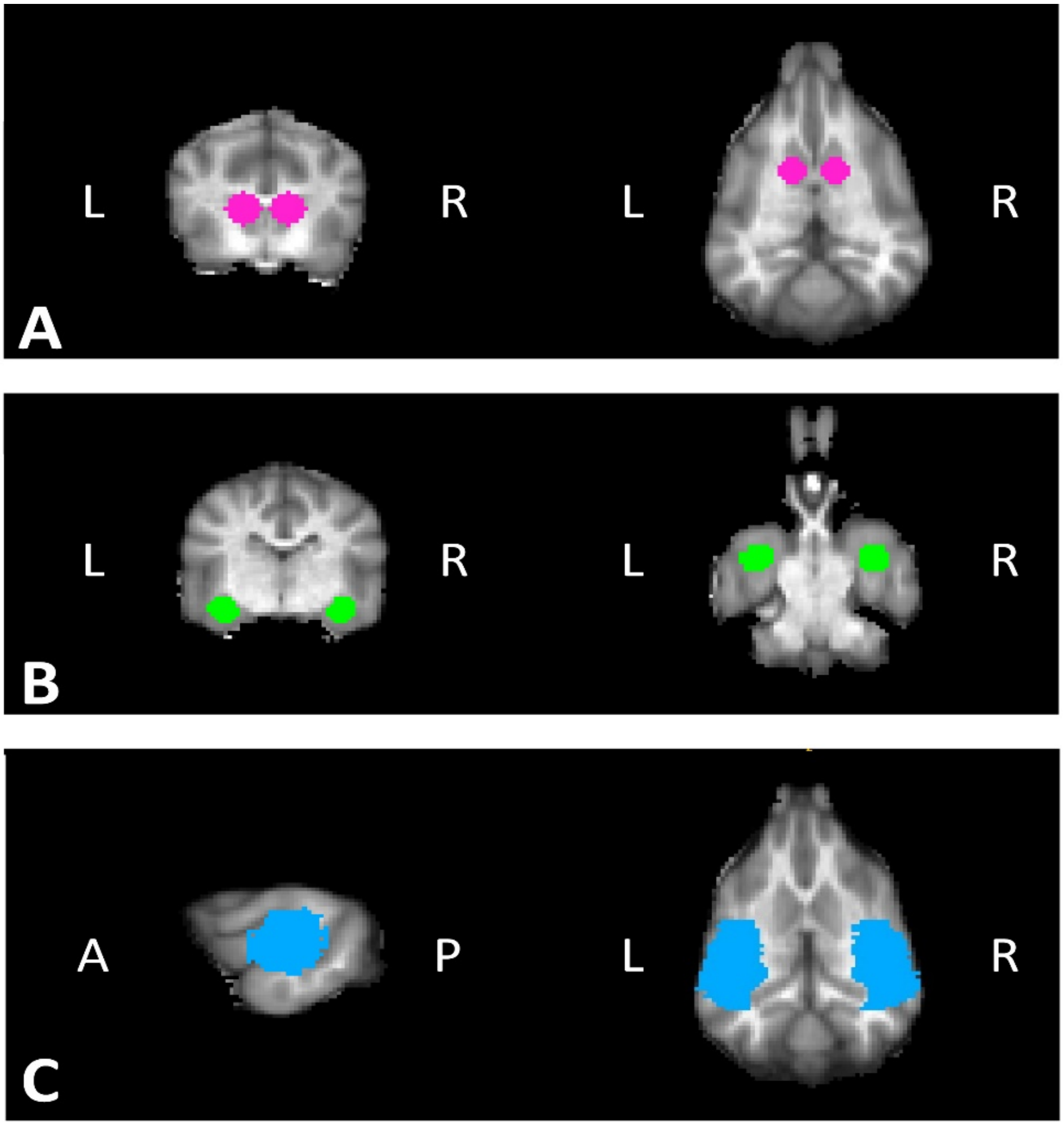

Figure 2. Regions of interest (ROIs) defined a priori. ROIs were drawn in individual anatomical space, example ROIs shown in template space here in transverse and dorsal views. **A)** Caudate nuclei have been shown to differentially respond to stimuli associated with reward and no-reward. **B)** Amygdalae have shown differential responding to stimuli associated with reward and no-reward, as well as arousal. **C)** Parietotemporal regions including primary auditory cortex respond to verbal stimuli, including nonwords. ROI is shown here in sagittal and dorsal views in template space.

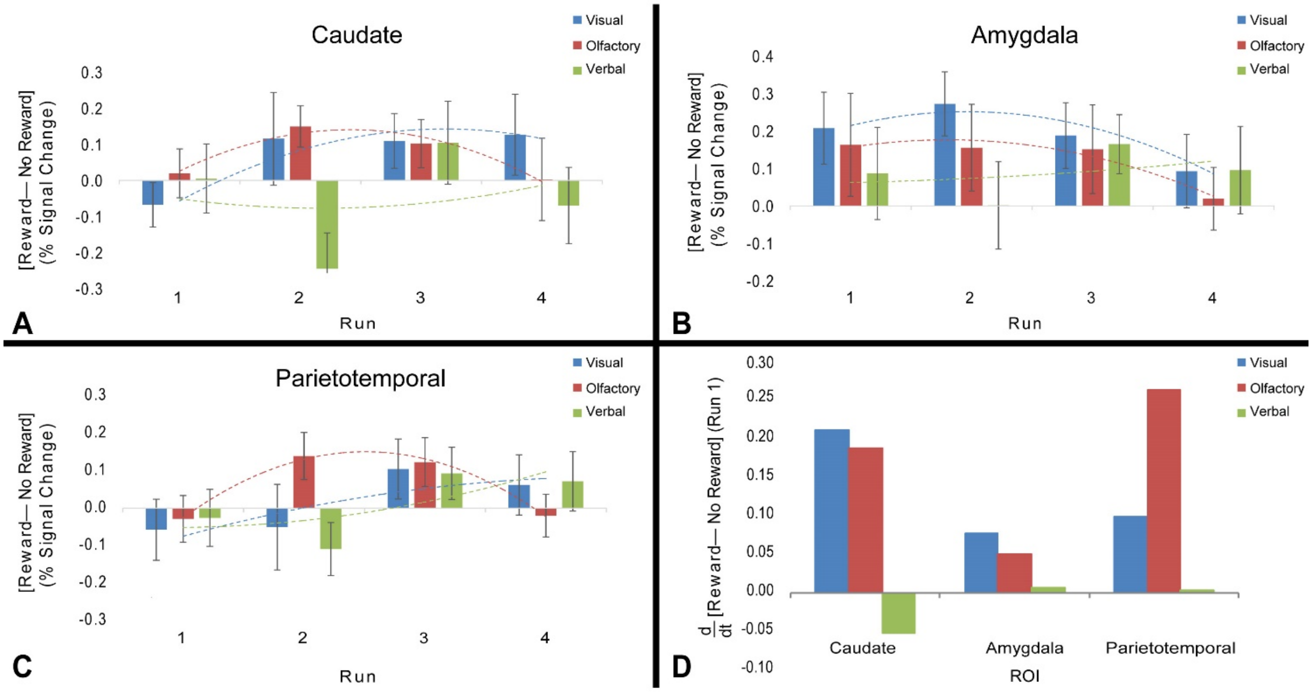

Figure 3. Percent signal change by ROI for the contrast of stimuli predicting Reward vs. No Reward. Unadjusted mean values across dogs by run and by modality (*blue* = visual, *red* = olfactory, *green* = verbal). Error bars denote the standard error of the mean across dogs for each modality and run. Lines denote second-order polynomial trend lines across all runs for each modality and ROI. Consistent with studies of reward learning, there were main effects of [Reward-No Reward] across all ROIs ($p < 0.001$), which was only marginally significantly different by modality ($p = 0.045$). There was a significant interaction between [Reward—No Reward] and ROI ($p = 0.014$), suggesting the magnitude of the effect was different in each region. All ROIs showed evidence of varying time course ($p = 0.018$), which differed by modality ($p = 0.006$), consistent with different rates of learning and habituation by modality. **A.)** Averaged beta values in the caudate show marked learning curves for visual and olfactory stimuli. **B.)** Averaged beta values in the amygdala show learning curves across all stimulus modalities, but verbal stimuli peak later than visual and verbal stimuli. **C.)** Averaged beta values in the parietotemporal area show weak learning effects for all modalities. **D.)** Comparison of initial learning rates for each modality for Run 1. Bars denote the temporal derivative (d/dt) of the polynomial fit for [Reward—No Reward] by modality and ROI. Across all three ROIs, percent signal change to visual and olfactory stimuli occur at a faster rate than verbal stimuli and is evident in the first few exposures.

Manuscript 2

Awake fMRI Reveals Brain Regions for Novel Word Detection in Dogs

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ABSTRACT

How do dogs understand human words? At a basic level, understanding would require the discrimination of words from non-words. To determine the mechanisms of such a discrimination, we trained 12 dogs to retrieve two objects based on object names, then probed the neural basis for these auditory discriminations using awake-fMRI. We compared the neural response to these trained words relative to “oddball” pseudowords the dogs had not heard before. Consistent with novelty detection, we found greater activation for pseudowords relative to trained words bilaterally in the parietotemporal cortex. To probe the neural basis for representations of trained words, searchlight multivoxel pattern analysis (MVPA) revealed that a subset of dogs had clusters of informative voxels that discriminated between the two trained words. These clusters included the left temporal cortex and amygdala, left caudate nucleus, and thalamus. These results demonstrate that dogs’ processing of human words utilizes basic processes like novelty detection, and for some dogs, may also include auditory and hedonic representations.

INTRODUCTION

Because dogs can learn basic verbal commands, it is obvious that they have the capacity for discriminative processing of some aspects of human language [1, 2]. For humans, words represent symbolic placeholders for a multitude of people, objects, actions, and other attributes. However, just because a dog can match a word with an action, like 'fetch,' does not mean that the dog understands the word has meaning in the same way humans do. For example, dogs may rely on other cues to follow verbal commands such as gaze, gestures and emotional expressions, as well as intonation [2-6]. This raises the question of what cognitive mechanisms dogs use to differentiate between words, or even what constitutes a word to a dog.

Part of the problem in studying word comprehension in dogs is the necessity of a behavioral response to demonstrate understanding. Some dogs can retrieve a named 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, and the border collie Rico, who demonstrated the ability to select a novel object among familiar objects based on a novel label [7-9]. But these dogs may have been exceptional. Few other dogs have been documented to have this level of expertise [10-13]. It may be that most dogs rely on simple mechanisms of discrimination – like novelty detection – coupled with other cues from the human to figure out an appropriate behavioral response.

The auditory oddball task, where subjects behaviorally discriminate between target and novel acoustic stimuli, is a well-established task used to measure the processing of target detection and decision-making in humans and nonhumans. The neural regions responsible for target detection and novelty processing not only include primary sensory areas associated with the stimulus modality, but also recruit broader areas such as the posterior cingulate, inferior and middle frontal gyri, superior and middle temporal gyri, amygdala, thalamus, and lateral occipital cortex [14-18]. This suggests that differentiating between target versus novel sounds requires primary auditory cortex as well as an additional attentional network to discriminate between competing sensory stimuli. At least one event-related potential (ERP) electroencephalogram study in dogs suggested similar novelty detection mechanisms might be at work, where dogs presented with auditory stimuli showed a greater amplitude of ERP to deviant tones relative to standard tones [19].

Recent advances in awake neuroimaging in dogs have provided a means to investigate many aspects of canine cognition using approaches similar to those in humans. 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 [20, 21]. These studies have furthered our understanding of the dog's neural response to expected reward, identified specialized areas in the dog brain for processing faces, observed olfactory responses to human and dog odors, and linked prefrontal function to inhibitory control [22-26]. In one fMRI study, dogs listened to human and dog vocalizations through headphones and showed differential activation within regions of the temporal and parietal cortex [27]. 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. However, a subsequent correction in which left and right were reversed raised questions about the interpretability of this finding [28].

To examine auditory processing in dogs, we used fMRI to measure activity in dogs' brains in response to both trained words and novel pseudowords. Over several months prior to scanning, owners trained their dogs to select two objects based on the objects' names. During the fMRI session, the owner spoke the names of the trained objects as well as novel pseudowords the dog had never heard before. If dogs discriminate target words from novel words as humans do, they should show differential activity in the parietal and temporal cortex in response to trained words relative to pseudowords [29-31]. In humans, this type of general semantic processing is associated with activity in a network comprised of the posterior inferior parietal lobe, middle temporal gyrus, fusiform and parahippocampal gyri, dorsomedial prefrontal cortex, and posterior cingulate gyrus [32]. In addition, if dogs use hedonic mechanisms to associate reward value with trained words, then differential activity should also be observed in the caudate.

MATERIALS AND 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.

Participants

Participants were 12 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, had previous experience with verbal commands from their owners, and had demonstrated the ability to remain still during training and scanning [20].

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

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 [8]. 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, to facilitate discrimination (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.

All dogs in the current study participated in training for previous fMRI experiments. As described in previous experiments [20-22, 33], 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.

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], though the number of distractor objects was sometimes increased during training to maximize discriminatory performance. 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%.

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 ranging from 35 to 128 days.

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 were spoken by the dog’s primary owner, who stood directly in front of the dog at the opening of the magnet bore. Both owners and dogs wore ear plugs, which reduced scanner noise by approximately 30 decibels, but allowed for intelligible human speech over the sound of the scanner. The spoken words were intelligible to the experimenters, who also wore ear plugs while next to the MRI during scanning, as well as human operators in the control room via the intercom. 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). Onset of words and objects were controlled by the simultaneous presentation and press of the button box by the experimenter marking the onset and duration of presentation. This was controlled manually by the experimenter during each dog's scan, as opposed to a scripted presentation as in human fMRI studies, because dogs may leave the MRI at any time and data for absentee trials would be lost.

An event-based design was used, consisting of four trial types presented semi-randomly: expected, unexpected, pseudoword, and reward. On expected 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 5 s and was subsequently allowed to interact with the object. During unexpected 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 corresponding 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 6 s inter-trial interval, and each dog received the same trial sequence (Fig. 2B). Each of three runs consisted of 26 trials, for a total of 78 trials. The trial types included: 30 expected (15 each of word1 and word2), 15 unexpected (7 or 8 of word1 and word2), 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 [20, 21]. 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 participant, 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 that best reflected their average position within the scanner. All volumes were aligned to the reference volume. 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 [33]. A high-resolution canine brain atlas was used as the template space for individual spatial transformations [36]. The atlas resolution was 1 mm x 1 mm x 1 mm. Thus voxel volumes are in mm^3.

General Linear Model

For *a priori* hypotheses, each participant'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). Nuisance regressors included motion time courses generated through motion correction, constant, linear, quadratic, and cubic drift terms. 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 included: (1) spoken word1; (2) spoken word2; (3) spoken pseudowords; (4) presentation of object1; (5) presentation of object2; (6) presentation of unexpected objects (novel object following either word1 or word2); and (7) presentation of novel objects following a pseudoword. The object on which each dog performed best during the day of the MRI scan as well as the object owners reported as being the preferred of the two was labeled as word1 and object1 when creating the GLM regressors. Stimulus onset and duration were modeled using the dmUBLOCK function, with the 5 utterances treated as a block.

Whole Brain Analysis

Contrasts focused on the dogs' response to words and pseudowords. Auditory novelty detection was probed with the contrast: [pseudowords – (word1 + word2)/2]. Low-level aspects of language processing (including acoustic and hedonic representations) were probed with the contrast [word1 – word2] and expectation violation with [novel objects – unexpected objects].

Each participant's individual-level contrast from the GLM was normalized to template space as described in [20, 22] via the Advanced Normalization Tools (ANTs) software [37]. Spatial transformations included a rigid-body mean EPI to structural image, affine structural to template, and diffeomorphic structural to template. These spatial transformations were concatenated and applied to individual contrasts from the GLM to compute group level statistics. 3dttest++, part of the AFNI suite, was used to compute a t-test across dogs against the null hypothesis that each voxel had a mean value of zero. All contrasts mentioned above as part of the GLM were included.

As there is spatial heterogeneity within fMRI data, the average smoothness of the residuals from each dog's time series regression model was calculated using AFNI's non-Gaussian spatial autocorrelation function 3dFWHMx –acf. The acf option leads to greatly reduced FPRs clustered around 5 percent across all voxelwise thresholds [38]. AFNI's 3dClustsim was then used to estimate the significance of cluster sizes across the whole brain after correcting for familywise error (FWE). Similar to human fMRI studies, a voxel threshold of $p \leq 0.005$ was used, and a cluster was considered significant if it exceeded the critical size estimated by 3dClustsim for a FWER ≤ 0.01, using two-sided thresholding and a nearest-neighbor of 1.

Multivoxel Pattern Analysis (MVPA)

In previous fMRI studies of the oddball task, it was noted that attentional differences occurring trial-by-trial may go undetected in the univariate analysis [16]. As an exploratory analysis, we used searchlight MVPA to identify regions potentially involved in the representation of words that were not captured in the univariate analysis. We were primarily interested in the representation of word1 vs. word2.

We used a linear support vector machine (SVM) for a classifier because of its previously demonstrated robust performance [39, 40]. Unsmoothed volumes were censored for motion and outlier count as in the univariate GLM. We then made a model for the unsmoothed data using AFNI's 3dDeconvolve stim_times_IM function. This model yielded trial-by-trial estimates (betas) for each repetition of word1 and word2, regardless of which object followed. Although it is common in the human literature to use each scan volume as a data point in MVPA (for training and testing), we have found this approach to be problematic with dogs, who move more than humans, resulting in spurious volumes that should be censored. Estimating the beta for each trial affords an additional level of robustness with less sensitivity to potential outlier volumes due to motion. As an additional check for outliers, masks were drawn of the left and right caudate on each dogs' T2-weighted structural image. Average beta values were extracted from both the left and right caudate for each trial of word1 and word2. Trials with beta values greater than |3%| were assumed to be non-physiological and were removed prior to MVPA. Finally, these trial-dependent estimates were then used as inputs to a whole-brain searchlight MVPA for each individual dog using PyMVPA2 [41]. The classifier was trained on the fMRI dataset for each dog by training on 2 runs and testing on the third using the NFoldPartitioner. We used the Balancer function to retain the same number of trials for word1 and word2 across training and testing for 100 repetitions. For the searchlight, we used a 3-voxel radius sphere. This yielded a map of classification accuracies throughout each dog's brain.

Given the difficulty in finding significant effects in small datasets using cross-validation and parametric methods, we used a permutation approach outlined by Stelzer et al. (2013) to determine the significance of any cluster of common voxels across dogs [42, 43]. Briefly, we permuted the order of attributes—but not their corresponding data—and ran the searchlight in individual space for all dogs. This created a null distribution of accuracies. The mean of these distributions was noted to be very close to 0.5, confirming that the classifiers wasn't biased or skewed. The cumulative distribution of that an accuracy ≥ 0.63 corresponded to the top 5% of voxels, and this was used as a cut-off threshold for the individual maps. These binarized maps were transformed into template space and the average computed across dogs. The resultant group map represented the locations of potentially informative voxels and served as qualitative representation of the relative consistency versus heterogeneity of word-processing in the dogs' brains. Somewhat arbitrarily, we only considered locations in which at least two dogs had informative voxels.

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. 3). 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 [$T(17.1) = 3.00$, $P = 0.008$] and that there was a significant difference in performance between word1 and word2 [$T(11) = 4.67$, $P < 0.001$].

Primary Auditory and Visual Activation

To confirm that the dogs clearly heard the words during scanning, a simple contrast subtracting activation to objects (trained and novel) from activation to words (trained and pseudowords) was performed. In human fMRI, the MRI operator may ask the participant whether they can hear auditory stimuli, which is not necessarily possible in dog fMRI, so this was included as a quality check. We opted for an unthresholded image not only to highlight the activation in bilateral auditory cortex but, just as important, to show what was not activated. Notably in the contrast [Words—Objects] positive activation was localized to the auditory cortex for words and negative activation for presentation objects in parietal cortex (Fig. 4), confirming that the dogs heard the words and saw the objects.

Whole Brain Analyses

Whole brain analysis of the contrasts of interest revealed significant activation only within the right parietotemporal cortex for the contrast [pseudowords – trained words]. With a voxel-level significance threshold of $P \leq 0.005$, the cluster size in the right hemisphere (839 voxels) was statistically significant at $P \leq 0.005$ after correction for whole-brain FWE (although activation appeared bilaterally) (Fig. 5). Whole brain analysis of the contrasts of [word1– word2] and [novel – unexpected] were not significant as no cluster survived thresholding at the voxel significance mentioned above.

MVPA

Because the univariate analysis of word1 vs. word2 did not reveal any region with a significant difference, we used MVPA to explore potential regions that may code for different representations of the words. The searchlight map of word1 vs. word2, which identified regions involved in the discrimination of the trained words, showed four clusters of informative voxels (Fig. 6): posterior thalamus/brainstem; amygdala; left temporoparietal junction (TPJ); and left dorsal caudate nucleus. Seven dogs shared informative voxels in or near the left temporal cortex that passed the 0.63 accuracy threshold (Fig. 7).

DISCUSSION

Using awake-fMRI in dogs, we found neural evidence for auditory novelty detection in the domain of human speech. The hallmark of this finding was greater activation in parietotemporal cortex to novel pseudowords relative to trained words. Thus, even in the absence of a behavioral response, we demonstrate that dogs process human speech at least to the extent of differentiating words they have heard before from those they have not. The mechanism of such novelty detection may be rooted in either the relatively less frequent presentation of the pseudowords (oddball detection) or the lack of meaning associated with them (lexical processing).

The activation observed in the parietotemporal cortex to pseudowords relative to trained words meets current standards of human fMRI analyses concerning up-to-date methods for cluster thresholds. Specifically, to address concerns raised by Eklund et al. (2016), present analyses for cluster inferences address the former Gaussian-shaped assumption about spatial structure in the residuals of fMRI data and provide more accurate false positive rates compared to previous methods [38, 44, 45]. As the identified cluster was significant at $P \leq 0.005$, corrected for whole-brain FWE, the result does not appear to be a false positive. However, as the study was limited to 11 participants, future studies with an increased number of participants could produce a more robust finding.

In humans, real words typically result in more activation than pseudowords, evoking activity in what has been called a general semantic network [32]. Although such activations are generally bilateral, they tend to lateralize more to the left and cluster around the angular gyrus, but that anatomical location is ill-defined and is sometimes called the temporoparietal junction (TPJ) or temporal-parietal-occipital cortex (TPO) [46]. It is clear that the nature of the task and the relative frequency of words and pseudowords can influence the relative activation to words and pseudowords. For example, stronger activation to pseudowords depends on whether the pseudoword resembles a known word or is so unlike known words as to prevent any semantic retrieval. When the pseudoword is similar to a known word, more processing has been observed in the superior temporal gyri, presumably to disambiguate it from known words [31, 47]. Thus, in dogs, the greater activation to the pseudowords could be due to the acoustic similarity between pseudowords and words that the dogs “knew” and their attempt to resolve the ambiguity. This would be a form of low-level lexical processing. However, previous research has shown that dogs can discriminate between altered phonemes of well-known commands [4], suggesting that it is unlikely that the dogs in our study were confused by acoustic similarity of words and pseudowords.

More likely, a novel word resulted in increased processing to facilitate learning the association with the novel object that followed. A dog’s behavioral bias for novelty is often described as an explanation for performance otherwise labeled as learning by exclusion [9, 48, 49]. As such, a dog may select a novel item because it is novel among other stimuli, but not because she has learned all other stimuli and associated a new word with the novel item. A bias for novelty would therefore be reflected in the dog's brain as with her behavior.

Auditory stimuli can be difficult to discriminate in the scanner. We used a continuous scanning protocol because that is what the dogs were accustomed to. The simple contrast of all words vs. all objects showed bilateral activation of the superior temporal lobe, indicating that the dogs heard something. However, the main effect of pseudowords vs. trained words showed that the majority of dogs discriminated well enough to tell the difference. The predominant location in the auditory pathway also suggests that the effect wasn't based on non-verbal cues from the handler (i.e. Clever Hans effect).

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 six months of age and differentiate between nouns prior to their ability to use verbs [50, 51]. 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 three dogs that consistently retrieved objects given a verbal referent [7, 8]. Additionally, those 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.

Although humans readily generalize the meaning of words to a variety of contexts, this may not be the case for dogs. The environment in which the dogs learned the words was different than both the testing and scanning environments [2]. In addition, although human fMRI language studies do not typically repeat the spoken word each trial, as is common in oddball paradigms, it was necessary for the dogs to make sure that they heard each word. Trials also did not include a condition in which a spoken pseudoword was followed by a trained object, or trials in which a trained object was mismatched to a trained word. These types of trials would have provided additional evidence for violation of expected semantic content; however, these types of trials have the potential to confuse the dogs and result in extinction of the words already learned. 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.

So what do words mean to dogs? Even though our findings suggest a prominent role for novelty in dogs' processing of human words, this leaves the question of what the words represent. One possibility is that the words had no further representation other than the relative hedonic value of the objects. While some dogs showed a behavioral preference for one object over the other, this preference was not reflected in whole brain analyses. Admittedly, the somewhat arbitrary designation of word1 / word2 and object1 / object2 could explain the nonsignificant results in the univariate analysis. Indeed, the MVPA of word1 vs. word2, which identified regions that classified the words above chance regardless of directionality, showed one cluster in the left caudate. However, the MVPA also identified clusters in the left TPJ, anterior temporal lobe and amygdala, and posterior thalamus. The TPJ was located just posterior to the region in the univariate analysis, which would take it out of the area of cortex associated with low-level acoustic processing. Its location appears similar to human angular gyrus. This region and its extension to the anterior temporal lobe look remarkably similar to the conceptual component of the general semantic network in humans [32, 52, 53]. If so, these could be potential sites for receptive word processing and even semantic representation in dogs, but future work would need to verify this.

Evaluating classifier performance for MVPA remains a complex task. We used MVPA as an exploratory analysis to identify brain regions that potentially discriminate between trained words across dogs. But classification using the whole brain may result in a high classification accuracy that is not generalizable across subjects. Indeed, the regions identified using MVPA were of marginal statistical significance, especially given the small sample size. Further, it should be noted that only a subset of dogs contained informative voxels in the TPJ region. Although all dogs had informative voxels somewhere in the brain, only seven dogs had informative voxels in the TPJ area. Thus, even though all the dogs were cleared for scanning by reaching performance criterion, they may have used different mechanisms to process the words. Like our previous fMRI studies, heterogeneity seems to be the rule [24, 33]. Even so, the accuracy of the classifier was not correlated with a dog's performance. This suggests that performance on such tasks may be influenced by factors other than word discrimination alone.

These results highlight potential mechanisms by which dogs process words. Word novelty appears to play an important role. The strong response of the parietotemporal region to pseudowords suggests that dogs have some basic ability to differentiate words with associations from those that do not. Future studies may reveal whether these representations remain in the auditory domain or whether such representations are invariant to modality.

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

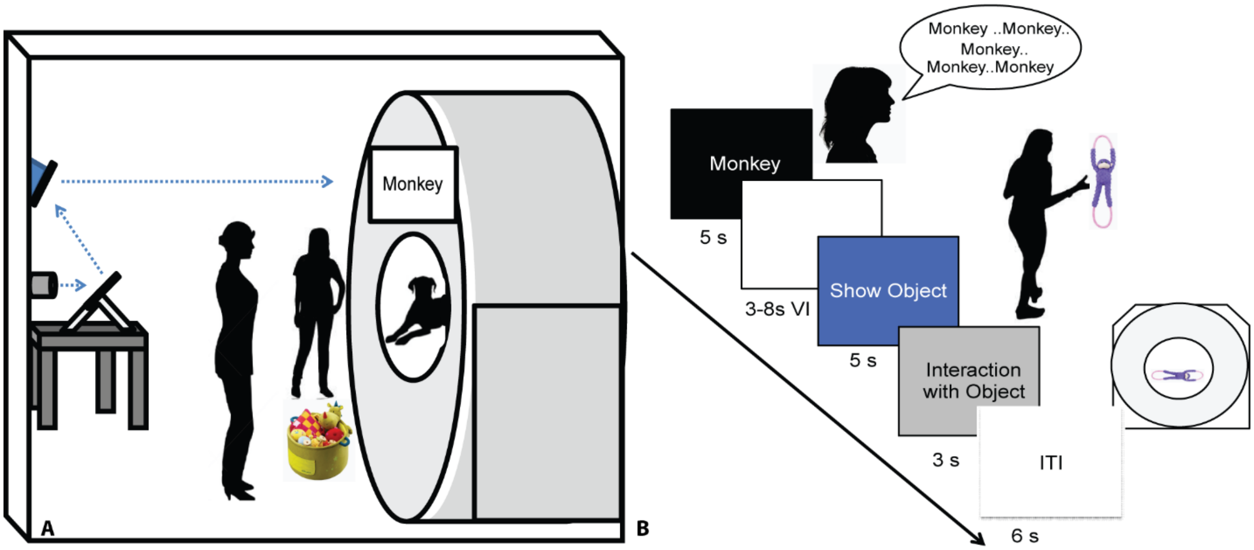

Figure 2. Experimental design. A) Experimental setup with mirror relay projected words onto MRI surface. Owner is facing the projected word and her dog while the experimenter controls the presentation of words and objects to the owner. **B)** Trial timeline indicating spoken word over 5 s, 3-8 s delay,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

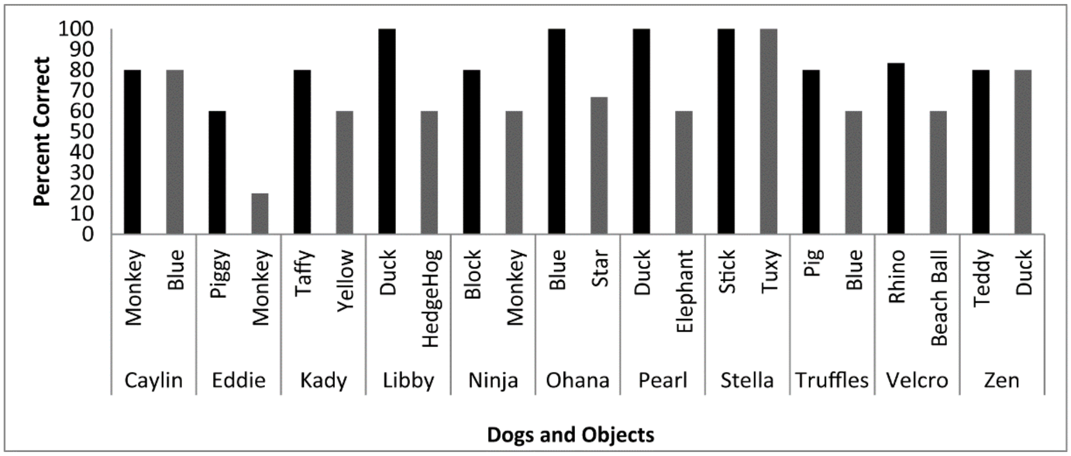

Figure 3. Individual performance on two object discrimination tests. Tests were conducted on the day of the fMRI scan. Each dog's Object 1 is in black, object 2 is in grey. All dogs performed significantly greater than chance, with the dog's greater performance or owner's report of their preference for one object over the other designating object 1.

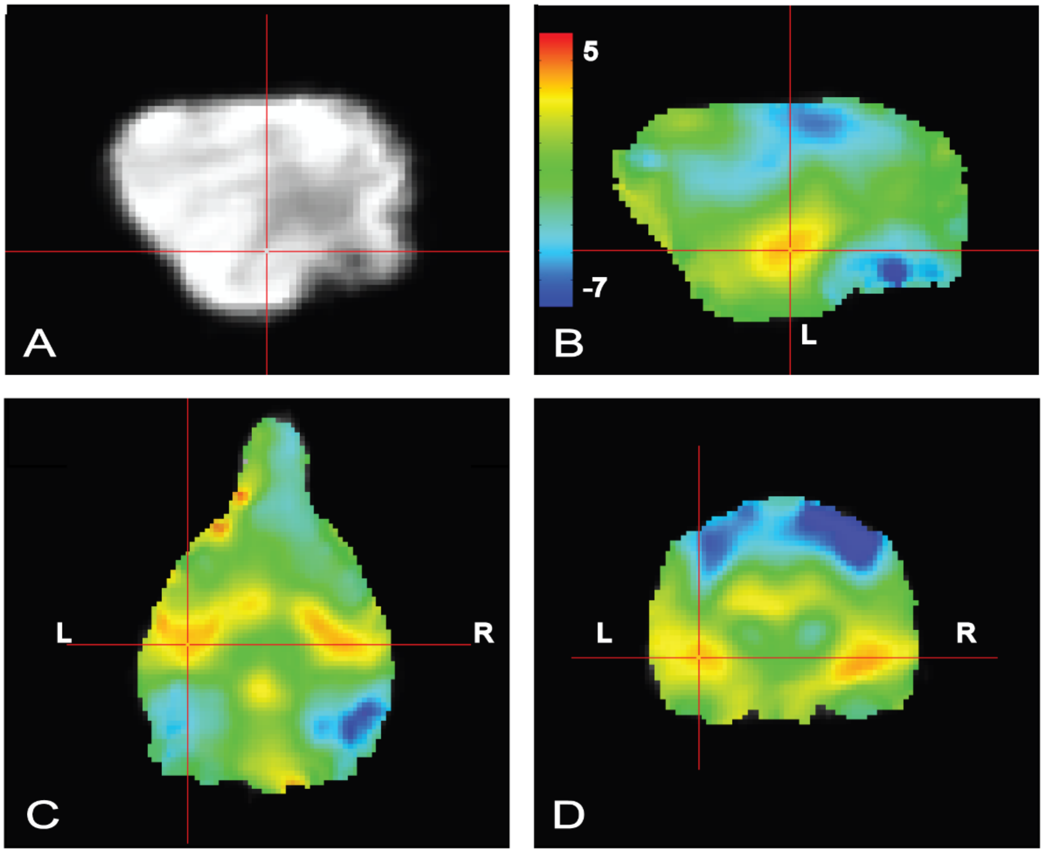

Figure 4. Whole brain group map showing unthresholded activation to all words versus all objects. **A)** Location of crosshairs in superior temporal lobe on average image of all dogs. **B)** Sagittal view of left hemisphere. Colors represent T-statistics. The primary auditory region extending into the parietotemporal area showed greater activation to words (*red*), whereas parietal and occipital areas showed greater activation to objects (*blue*). **C)** Dorsal view. **D)** Transverse view.

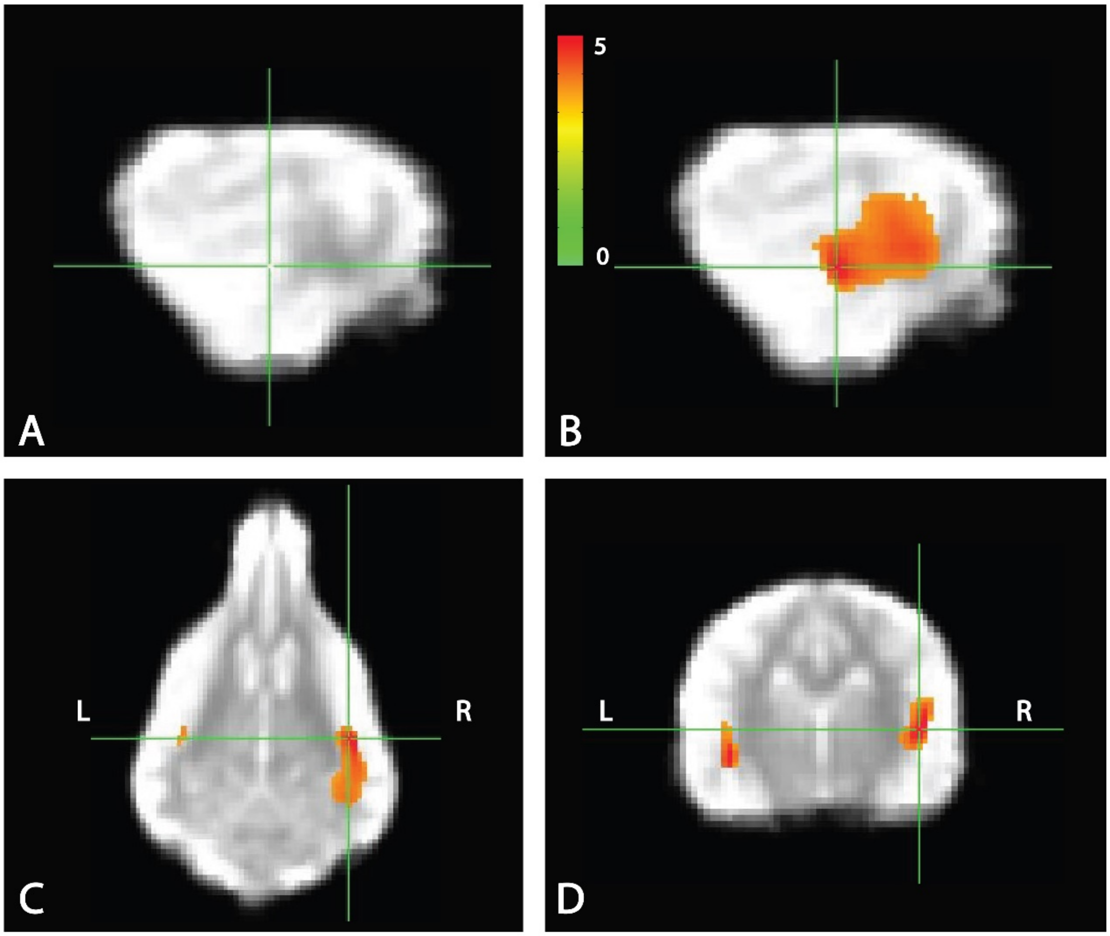

Figure 5. Whole brain response to [pseudowords – words] contrast. Whole brain analysis revealed significant activation within a parietotemporal region including primary auditory cortex and neighboring regions. **A)** Location of crosshairs on average image of all dogs without overlay. **B)** Sagittal view of right hemisphere. Colors represent T-statistics. With a single voxel significance of 0.005, the clusterwise significance (Right: 839 voxels; Left: 43 voxels) corrected across the whole brain was *P* = 0.005 for the right hemisphere, though activation seemed bilateral. **C)** Dorsal view. **D)** Transverse view.

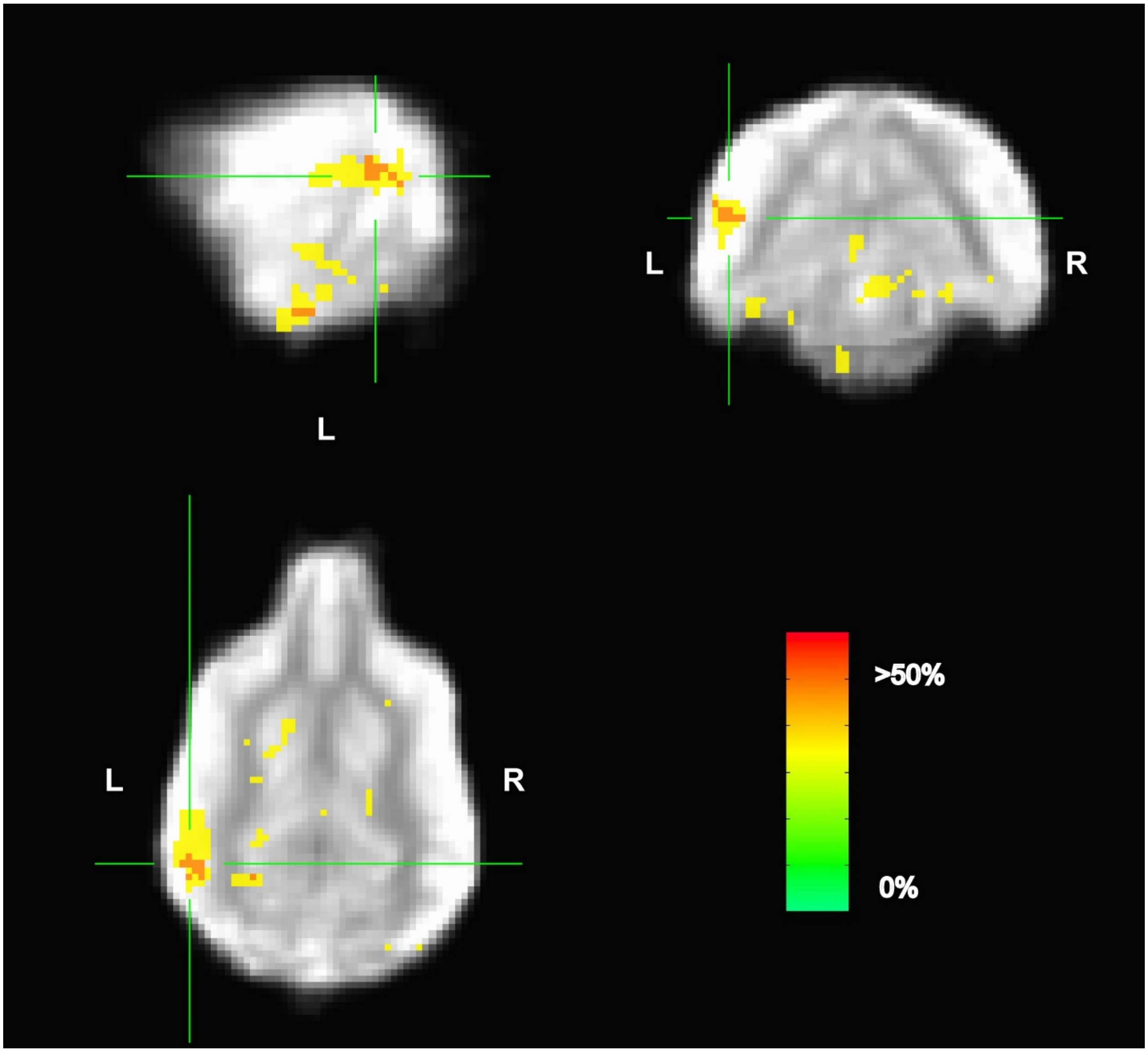

Figure 6. Aggregate performance of searchlight MVPA classifier for word1 and word2 across dogs. Color intensity indicates fraction of dogs with informative voxels at each location. The image is thresholded such that only voxels that were informative for more than one dog are shown. This map showed four clusters: posterior thalamus/brainstem; amygdala; left temporoparietal junction; and left dorsal caudate nucleus. The temporoparietal junction appears similar to human angular gyrus and could be a potential site for receptive language processing in dogs.

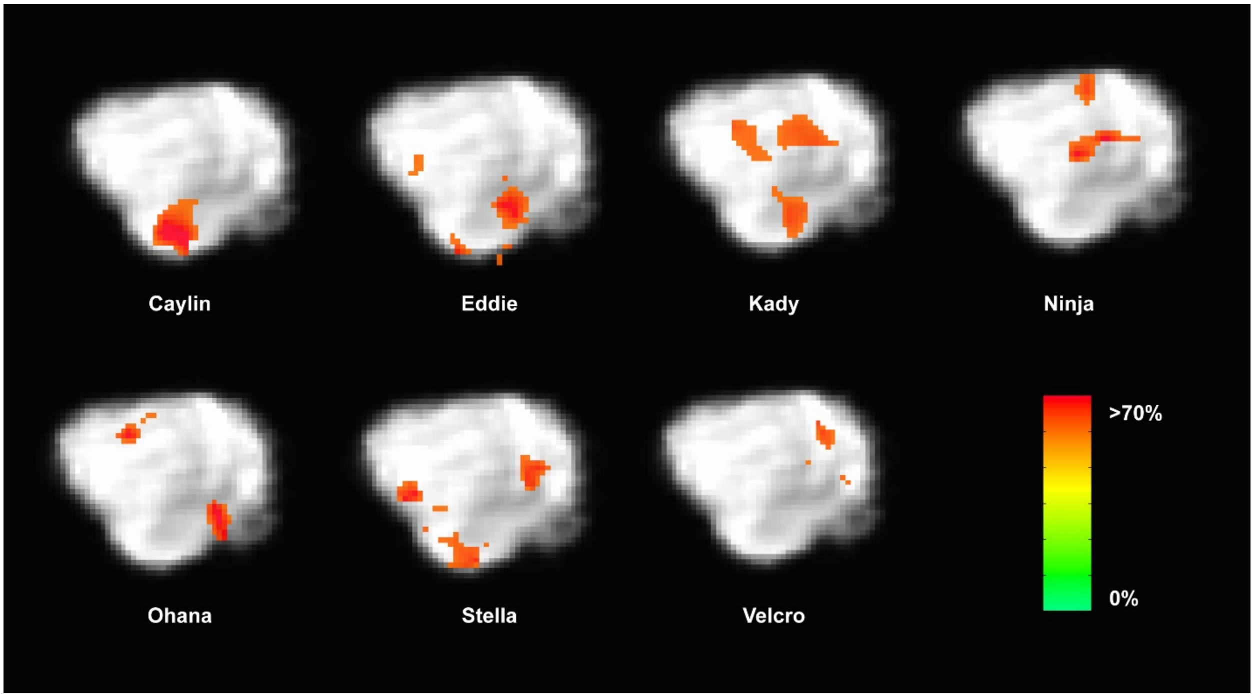

Figure 7. Dogs with informative voxels for word1 and word2 in the left temporal and parietal lobes. Color intensity indicates classification accuracy at each location, thresholded ≥ 0.63. Seven dogs displayed clusters in the left temporal and parietal lobes, suggesting some heterogeneity in the location underlying word discrimination.

Manuscript 3

How Dogs Perceive Two-Dimensional and Three-Dimensional Objects

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ABSTRACT

Dogs have access to many forms of screens and touch pads, with owners even subscribing to dog-directed content on YouTube. However, we do not know what dogs experience when viewing two dimensional (2D) stimuli or if they use pictures as referents for real world three dimensional (3D) stimuli. Here, we used awake fMRI of 16 dogs in three studies to examine the neural mechanisms underlying dogs' perception of dimensional objects. In the first study, we defined object processing regions in the dog brain using videos of objects compared to videos of faces. In the second study, we examined whether the affordances from training the dogs to manipulate objects with either their mouth or paw affected neural responses within object processing regions of the dog brain. In the third study, we explored potential differences in neural activation between 2D and 3D versions of objects after dogs were trained on one of the two versions. We identified regions of the dog brain that respond to objects in temporo-occipital regions and parietal regions, which are functionally analogous to the lateral occipital complex (LOC) and anterior parietal sulcus (aIPS) in humans. Activation within reward processing regions and parietal cortex of the dog brain to 2D and 3D versions of objects was also mediated by their experience, as dogs trained on one dimensionality showed greater activation to the dimension with which they were more familiar. Dogs also exhibited neural biases for the objects they associated with a mouth affordance over objects that they associated with a paw affordance. Together, our results shed light on dogs' neural biases for processing objects and warn against the assumption that dogs use pictures or videos as referents for real world stimuli.

INTRODUCTION

Studies of canine cognition frequently rely on pictures to test dogs' ability to discriminate between objects, species, or faces [1-7]. Visual stimuli for these studies are utilized because they are easy to obtain from studies on humans and nonhuman primates and are easy to implement in laboratory settings. But dogs may not perceive these visual stimuli as humans do, calling into question whether images are appropriate stimuli for the study of dog cognition.

Visual stimuli are often selected without considering the nature of dogs' visual perception[8, 9]. Dogs have a higher flicker fusion rate than humans, meaning that they process items within movies at a different rate than humans and may notice a gap or flicker between frames. Dogs also have a visual streak as opposed to a fovea (as in primates), causing increased sensitivity to stimuli in the periphery of dogs' visual field rather than in the center. This means that displaying a picture or playing a video to a dog may not accurately reflect what a dog sees because they may focus on different aspects of the video than we do [8, 10]. Further, there is little evidence that dogs can use two-dimensional (2D) stimuli to represent three-dimensional (3D) or real-world stimuli, (i.e. 2D stimuli may not capture a dog's attention or have the same meaning as they do to humans).

Studies that use pictures share an underlying assumption that, like humans, dogs perceive 2D stimuli such as faces as identical to real 3D faces. Studies examining face perception in dogs propose that dogs integrate both auditory and visual information when perceiving people, suggesting that pictures alone may not be sufficient to recognize a familiar face [11, 12]. Dogs also discriminate between pictures of human facial expressions and between pictures of familiar and strange dogs or humans [1-4, 6, 13], and following substantial training, dogs show the ability to follow commands presented by humans through video projection [14]. One study reported dogs' use of duplicates of the objects or miniature versions as referents to retrieve the corresponding objects, concluding that dogs can use iconic representations [15]. However, the same dogs did not perform well using pictures versions of the objects. Despite this widespread use of 2D visual stimuli in canine cognition, studies have not shown that dogs use 2D stimuli as referents for real world stimuli.

Humans' ability to abstract from two-dimensional (2D) to three-dimensional (3D) space develops during infancy, and requires some experience with 2D stimuli [16-18]. The ability to abstract from 2D to 3D versions of objects, however, is not uniquely human. Many nonhuman species show evidence of behavioral transfer from pictures or videos to objects, pictures of food, or conspecifics [16, 19, 20]. In nonhumans, successful transfer between pictures and objects occurs when they are trained on a set of either pictures or objects and show transfer of a learned response to the opposite set. There is little evidence for 2D to 3D transfer happening naturalistically in any nonhuman species, and recognition between pictures and objects does not mean that the animal has abstract knowledge of objects, or that they equate pictures and real world objects [21, 22].

Studies of abstraction from 2D to 3D in nonhumans are difficult to interpret because behavioral measures are used to test whether an animal has formed a mental representation. Behavioral measures also make it difficult to rule out alternate explanations for successful abstraction like color/shape matching or “Clever Hansing” [22]. Studies of dogs cognition using objects have demonstrated behavioral evidence for the formation of relational concepts, object permanence, or object recognition [8, 23-29]. These studies rely on the dog performing the correct indication behavior, tracking the location of an object, or even using a touch screen in the presence of a handler. Yet studies of dog behavior run the risk of overinterpretation, and because of this, it’s important to look for other sources of evidence, like modeling the methods of human visual perception studies to examine dogs’ perception of object stimuli.

Using functional magnetic resonance imaging (fMRI), regions of the primate brain have been identified as selective for processing specific types of visual stimuli, including the fusiform face area (FFA) for processing faces or the lateral occipital complex (LOC) for processing objects [30-35]. Regions in the dorsal pathway, including parietal regions, are thought to process the where and how of interacting with object stimuli (affordances), whereas the ventral pathway, including temporal regions, is thought to process the identity of the object. Yet these fMRI studies have a similar caveat to studies of dog cognition: they too rely on 2D visual stimuli as proxies for real-world stimuli.

Object processing regions of the human brain respond differently to 2D and 3D versions of stimuli. An fMRI study that directly compared neural activation within the LOC to real world objects and 2D versions of the same objects found that the brain does not respond to the two versions of stimuli in the same way [36]. Real objects also prompt greater attention and memory retrieval than 2D images, and elicit goal-directed actions whereas 2D images do so to a lesser degree [37, 38]. Goal directed actions such as grasping involve dissociable object-affordance pairings that are difficult to generalize to 2D versions of objects because 2D versions lack the same binocular cues or proprioceptive feedback. Differences in humans’ perception of 2D and 3D versions of the same objects are therefore thought to be due to the affordances associated with the object [39-41].

As in human studies of vision, fMRI can be used to elucidate the neural mechanisms underlying dogs' perception of objects. FMRI studies of awake dogs have increased in complexity and duration paralleling human fMRI studies. Canine studies show that stimulus-reward associations acquired prior to or during scanning are learned at different rates due to neural biases within the reward-processing regions of the brain, such as the caudate and amygdala [42, 43]. Dogs also process familiar human words associated with objects in similar language-processing regions of humans, like the temporal-parietal junction, and show greater activation to novel words versus familiar words [44]. As in human imaging, functional localizers have also revealed areas of dogs' occipital cortex selective for processing human and dog faces [45-47]. Together these studies show that activation within areas of the dog brain can be used to predict perceptual or behavioral biases when processing of visual stimuli. Here, we performed a series of three fMRI experiments to address different aspects of visual perception in dogs.

In Experiment 1, we performed a functional localizer for objects using the same movies that are used in human studies. dogs participated in a second MRI scan encompassing a functional localizer to identify regions specific to object perception. The functional localizer included movies of novel faces, novel objects, the 3 trained objects, and scrambled images. If dogs form object representations based on shape, then activation in the dog brain to objects will be in areas homologous to the LOC. However, if object representations are grounded in affordances, then neural activation may extend for the affordance-associated objects into parietal regions of the "where" or dorsal stream. Like humans, neural activation to 2D version of object stimuli associated with affordances will be less than to the 3D objects because 2D stimuli do not have apparent affordances.

In Experiment 2, we used fMRI to test the effect of affordance on object representation. Prior to the MRI scan, we trained 12 dogs to grasp one object with their mouth and to touch one object with their paw. A third object was presented to the dog but not interacted with, to serve as a control. During the scan session, dogs were presented all three trained objects by their owners. Neural activation was compared during the presentations of all three objects within functionally defined regions associated with object perception and regions for planning motor actions, such as the anterior intraparietal sulcus (aIPS). If dogs discriminate between object stimuli based on their affordances (e.g. fits in mouth), then we would expect there to be differential neural activation in parietal regions between the two objects based on their trained affordances.

In Experiment 3, we used fMRI to measure activity in dogs' brains in response to both objects and pictures of the objects. Prior to scanning, 15 dogs were split into two groups. Dogs in the first group were trained on two 3D object stimuli, one of which was associated with reward and the other with nothing. Dogs in the second group were trained on two 2D picture stimuli, and like before, one was associated with reward and the other with nothing. During the fMRI session, dogs from both groups were presented both the picture stimuli and object stimuli. If dogs equate 2D and 3D stimuli, then they should show no difference in neural activity between the object and the picture in areas such as the LOC. Further, if hedonic mechanisms facilitate abstraction from 2D to 3D versions of object stimuli associated with reward, then dogs will show greater neural activity for the trained reward stimulus than the no reward stimulus, and similar trends in activation to the untrained stimuli.

MATERIALS AND METHODS

Participants

Participants for all three studies were a subset of 19 pet dogs volunteered by their Atlanta owners for fMRI training and fMRI studies [43] (Table 1). Each dog had previously completed two or more scans for the project and had demonstrated the ability to participate in MRI scans. Not all dogs were available to participate in each of the three studies (see Table 1.). 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 (Protocols DAR-2002879-091817BA and DAR-4000079-ENTPR-A), and all owners gave written consent for their dog's participation in the study.

Table 1. Dogs (N=19) and participation in experiments.

Dog	Breed	Sex	Object Localizer	Affordances	2D to 3D	Mouth object	Paw object	S+
BhuBo	Boxer mix	M	1	1		Pin	Die	
Caylin	Border collie	F	1	1	1	Pin	Die	3D Giraffe
Daisy	Pitbull mix	F	1		1			3D Giraffe
Eddie	Labrador Golden mix	M	1		1			2D Whale
Kady	Labrador	F			1			3D Whale
Kaper	Duck Trolling Retriever	M		1		Pin	Whistle	
Koda	Pitbull mix	F	1	1	1	Whistle	Pin	2D Giraffe
Libby	Pitbull mix	F	1		1			3D Whale
Loki	Cocker spaniel	M	1	1		Whistle	Pin	
Mauja	Cattle dog mix	F			1			2D Whale
Ohana	Golden Retriever	F	1		1			3D Whale
Oliver	Border collie Beagle mix	M	1	1	1	Die	Whistle	2D Whale
Pearl	Golden Retriever	F	1	1	1	Whistle	Pin	2D Giraffe

Rookie	Goldendoodle	M	1	1		Die	Whistle	
Tallulah	Carolina dog	F	1	1	1	Pin	Die	2D Whale
Truffles	Pointer mix	F	1	1	1	Pin	Die	3D Giraffe
Tug	Portuguese Water dog	M			1			3D Giraffe
Velcro	Viszla	M	1	1		Whistle	Pin	
Zen	Labrador Golden mix	M	1		1			2D Giraffe
Zoey	Goldendoodle	F	1	1	1	Whistle	Pin	3D Giraffe
N			**16**	**12**	**15**			

Dog's names, breed, sex, and participation in experimental studies (indicated by 1) are listed. Grey denotes a dog participated in all 3 studies.

General Experimental Design

The experimental design was similar to previous dog fMRI studies that examined preference using visual stimuli associated with food or social reward [42]. Briefly, dogs entered and positioned themselves in custom chin rests in the scanner bore. All scans took place in the presence of the dog's owner, who stood or sat directly in front of the dogs throughout the scan at the opening of the scanner bore and delivered all rewards (hot dogs) to the dog. An experimenter was stationed next to the owner, out of view of the dog, where the experimenter controlled the timing and presentation of stimuli to the dogs via a four-button MRI-compatible button box. The onset and offset of each stimulus were timestamped by the simultaneous press of the button box by the experimenter.

Imaging

Scanning was conducted with a Siemens 3 T Trio whole-body scanner using procedures described previously [43, 44, 48, 49]. 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 = 1260 ms, flip angle = 70˚, 64 x 64 matrix, 2.5 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. Four runs of up to 400 functional volumes were acquired for each subject, with each run lasting about 9 minutes. Following functional scans, 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).

Preprocessing

Preprocessing was the same as described in previous studies. Briefly, preprocessing of the fMRI data included motion correction, censoring, and normalization using AFNI (NIH) and its associated functions. A hand-selected reference volume for each dog that corresponded to their average position within the magnet bore across runs was used for two-pass, six-parameter rigid-body motion correction. Aggressive censoring removed unusable volumes from the fMRI time sequence because dogs can move between trials and when consuming rewards. Data were censored when estimated motion was greater than 1 mm displacement scan-to-scan and also based on outlier voxel signal intensities. Smoothing, normalization, and motion correction parameters were identical to those described in previous studies [43]. EPI images were smoothed and normalized to %-signal change with 3dmerge using a 6mm kernel at full-width half-maximum. The Advanced Normalization Tools (ANTs) software was used to spatially normalize the mean of the motion-corrected functional images [50] to the individual dog's structural image. We also performed a nonlinear transformation from each dog's structural image to a high-resolution canine brain atlas, developed from Labrador Retrievers from a previous study [51].

Object Localizer

Experimental Design

Stimuli were presented using Python 2.7.9 and the Psychopy Experiment library. Each stimulus block was manually triggered by an observer at the rear of the magnet. This manual triggering ensured that the dog was properly stationed at the beginning of each stimulus block.

A blocked fMRI design was used in which the dogs viewed either movie clips (dynamic stimuli). On average, each dog completed three dynamic stimulus runs. In the dynamic runs, dogs were presented with 3-s color movie clips of human faces, novel objects (toys), trained objects (from affordance study), and scrambled objects. The scrambled object movies were constructed by dividing each object movie clip into a 15 by 15 box grid and spatially rearranging the location of each of the resulting movie frames. There were seven movie clips for each category such that each block was 21 s. Each run contained two sets of four consecutive stimulus blocks in palindromic order (e.g., novel objects, faces, scrambled, trained objects, trained objects, scrambled, faces, novel objects), to make two blocks per stimulus category per run. Each run stimulus block had a delay of 10 s, where dogs were fed intermittently between blocks, such that each run was approximately 6 minutes.

Analyses

For each subject, a General Linear Model was estimated for each voxel using 3dDeconvolve. Motion time courses were generated through motion correction, and constant, linear, quadratic, cubic, and quartic drift terms were included as nuisance regressors. 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 for each experiment were modeled using AFNI's dmUBLOCK and stim_times_IM functions as follows: (1) faces, (2) novel objects, (3) trained objects, and (4) scrambled objects. This function created a column in the design matrix for each trial, allowing for the estimation of beta values for each trial. A mask was drawn in functional space for each dog in the cerebellum, which was used to censor the data further by removing volumes where the beta values extracted from the cerebellum were greater than an absolute value of 3 percent signal change for each trial. These beta values were assumed to be beyond the physiologic range of the BOLD signal. Object-selective regions, such as LOC, were identified with the contrast [novel objects —scrambled] and [novel objects-faces].

Each dog's object-specific region was defined by thresholding the voxel threshold of the statistical map for the [novel objects—faces] contrast until the number of voxels in each ROI was approximately 40 voxels or less, comparable to the size of the object processing regions defined in human fMRI studies (Table 2).

Affordances

Object-Affordance Training

Dogs were randomly assigned three objects, with two objects associated with an affordance during training (mouth or paw), and the third object being equally familiar but without a trained affordance (Fig 1). The objects were a 2.5-inch green foam cube, a 6-inch wooden train whistle, and an 8-inch white plastic bowling pin. These objects were chosen because they were novel to the dog and discriminable based on shape, texture, and color.

Training began on the affordance that owners reported to be their dogs preferred method of interacting with objects. The neutral object was present during all training but not interacted with by the dog such that the object was familiar but unassociated with an affordance.

Owners trained the paw affordance by first familiarizing the dog with the *paw* object. Owners then practiced the verbal command paw, high-five, or shake (whichever was known by the dog) with the dog using positive reinforcement (food or praise). The verbal command was then paired with the object held by the owner as the desired target for the dog's paw. Positive reinforcement was used to reinforce dogs touching the target object in response to the verbal command. Owners then continued presenting the object to the dog without a verbal command. Dogs were positively reinforced when they placed their paw on the target object when the object was presented. When one object-affordance was successfully trained, dogs began training on the other object, alternating training every other day between old and new.

Owners trained the mouth affordance familiarizing the dog with the *mouth* object. Owners then practiced the verbal command take, fetch, or touch (whichever was previously known) paired with the object while it was held or tossed by the owner to the dog. Owners shaped dogs' responses using positive reinforcement (food or praise) when the dog touched the target object in response to the command. Dogs were then reinforced only when they placed their mouth on the target object. Owners then continued to present the object to the dog without a verbal command. Dogs were positively reinforced when they grasped the object with their mouth when the object was presented.

To test dogs' acquisition of the object-affordance association, all three objects were randomly presented by the owner. The dog was positively reinforced when they performed the correct trained affordance on the corresponding object. A training criterion was set such that dogs behaviorally discriminated between the two trained objects on 8 out of 10 trials for each object in one session prior to scanning.

Experimental Design

Ten dogs that passed the training criterion participated in the experiment. Dogs positioned themselves within the MRI bore using the custom chin rests at the beginning of the session. Prior to scanning, a warm-up session included the semi-random presentation of the objects from training for 4 s by the owners. Dogs were reinforced by the owner with verbal praise and/or a treat when, after viewing the object, they performed the correct affordance associated with the object when the object was offered.

An event-based fMRI design was used with the experiment separated into 3 runs. Each run consisted of 12 blocks in a randomized order. In 8 presentation blocks, dogs were presented the objects by the owners six times in quick succession in a semi-random order for a duration of 4 s per object and one 4 s null instance in which nothing was presented. Each object was presented 48 times in total during the presentation blocks across the 3 runs. In 4 affordance blocks, the dogs were presented with either of the 2 objects associated with affordances for 4 s, then allowed to interact with the object. Each of the affordance objects was presented twice per affordance block, for a total of 8 opportunities to interact with the object per run. If the dog performed the correct affordance (e.g. mouth or paw) associated with the object, they were reinforced with food or verbal praise by the owner. Experimenters noted during each affordance trial whether the correct affordance was performed on the object. Each of the 3 runs lasted approximately 11 minutes. Dogs exited the scanner between runs for a short rest or water.

Analyses

Each subject's motion-corrected, censored, smoothed images was analyzed within a general linear model (GLM) for each voxel in the brain using 3dDeconvolve (part of the AFNI suite). Motion time courses were generated through motion correction, and constant, linear, quadratic, cubic, and quartic drift terms will be included as nuisance regressors. 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 for each experiment were modeled using AFNI's dmUBLOCK and stim_times_IM functions as follows: (1) paw object; (2) mouth object; and (3) familiar object. Data were censored for outliers as described above for the contrasts of interest.

Acquisition of the trained object-affordance associations for the real objects was probed with the contrast [trained objects—neutral object]. Discrimination between the objects based on the trained affordances was probed with the contrast [paw object—mouth object]. Transfer of the trained object-affordance associations to the movie versions of objects was examined with the contrast [trained objects—novel objects].

2D to 3D

Experimental Design

In each session dogs were presented with two objects (a stuffed giraffe and a stuffed whale) and two life-sized cut-out pictures posted on foamboard of the objects (Fig 2). Each stimulus was attached to a three-foot dowel that the experimenter used to present the stimuli to the dog while inside the scanner bore. Neither object had been encountered before by the dogs. Dogs were semi-randomly split into two conditions prior to scanning, 8 in the object condition and 7 in the picture condition. Prior to the first run, dogs were trained on the stimulus-reward associations (10 reward, 10 no-reward) based on their assigned condition. Prior to runs two through four, dogs were again trained on the stimulus-reward associations (5 reward, 5 no-reward). Following each run, dogs would exit the scanner and rest or drink water.

Dogs in the object condition were trained on object stimuli and were semi-randomly assigned the whale or giraffe as the reward stimulus. The presentation of the reward object (giraffe or whale) was immediately followed by the delivery of a food reward, and presentation of the other object was immediately followed by nothing. In the picture condition dogs were trained on picture stimuli and were semi-randomly assigned the whale or giraffe as the reward stimulus. The presentation of the reward picture (giraffe or whale) was immediately followed by the delivery of a food reward, and the other picture was immediately followed by nothing. Training on the conditioned stimuli occurred prior to each run when the dog was positioned in the scanner bore, but before scan acquisition. During scan acquisition, no stimuli were followed by the delivery of a food reward, so that dogs could not discriminate between objects and pictures based solely on food reward. Food rewards were presented by the owner randomly throughout the scan session between presentations of the stimuli.

An event-based design was used, consisting of trained reward and trained no-reward trial types, as well as symbolic reward and symbolic no-reward trial types. Trained reward and trained no-reward trials consisted of the two conditioned stimuli associated with food reward prior to scanning (e.g. objects for half of the dogs, pictures for the other half). Symbolic reward and symbolic no-reward trials consisted of the two untrained stimuli (e.g. pictures for dogs trained on objects, objects for dogs trained on pictures). On all trials, a stimulus was presented for a 5 s duration, followed by nothing. Trials were jittered to randomize presentation order and were separated by an inter-trial interval using Waver (part of the AFNI suite) to create the ideal reference function for each trial type, followed by 3dDeconvolve (part of the AFNI suite) to find the ideal inter-trial interval between each stimulus presentation. Each dog received the same trial sequence.

A scan session consisted of 4 runs, lasting approximately 9 minutes per run. Each run consisted of 25 trials (5 trained reward, 5 trained no-reward, 5 symbolic reward, 5 symbolic no-reward, and 5 food rewards), for a total of 100 trials per scan session. No trial type was repeated more than 4 times sequentially, as dogs could habituate to the continued presentation of a stimulus.

Analyses

Each subject's motion-corrected, censored, smoothed images were analyzed within a general linear model (GLM) for each voxel in the brain using 3dDeconvolve (part of the AFNI suite). Task related regressors for each experiment were modeled using AFNI's dmUBLOCK and stim_times_IM functions and were as follows: (1) trained reward stimulus; (2) trained no-reward stimulus; (3) symbolic reward stimulus; (4) symbolic no-reward stimulus. This function created a column in the design matrix for each trial, allowing for the estimation of beta values for each trial. Data were censored for outliers as described above for the contrasts of interest.

Acquisition of the trained stimulus-reward association was probed with the contrast [trained reward— trained no reward)]. Transfer of the trained reward and no-reward association to the untrained stimuli was probed with the contrast [symbolic reward—symbolic no reward)]. A direct association between the trained and untrained reward stimuli was tested with the contrast [trained reward – symbolic reward]. The contrast [all_3D—all_2D] was performed to test for perceived differences between all 3D and all 2D stimuli, regardless of training. The between trained stimuli and representative stimuli was assessed with the contrast [(trained reward + trained no-reward)—(symbolic reward + symbolic no-reward)] as well as the interaction between reward and no reward stimuli and familiarity with the contrast [(trained reward — trained no reward)—(symbolic reward —symbolic no reward)].

Region of Interest (ROI) Analysis

As our interest was based on the dog's response to trained stimuli versus untrained stimuli, quantitative analyses based on the imaging results used activation values in the canine brain area previously observed to be responsive to visual stimuli [42]. Anatomical ROIs of the left and right caudate nuclei were defined structurally using each dog's T2-weighted structural image of the whole brain. ROI-based analyses were performed in individual, rather than group space.

Beta values for the contrasts comparing the change in activation to reward and no reward stimuli for trained (20 reward trials, 20 no-reward trials) and symbolic stimuli (20 reward trials, 20 no-reward trials) were extracted from the caudate ROIs in the left and right hemispheres. Beta values no greater than an absolute four percent signal change were removed prior to analyses (assuming that these were beyond the physiologic range of the BOLD signal). The remaining beta values were compared across presentations of the stimuli by training (either objects or pictures, as well as to test for any hemispheric differences. We used the mixed-model procedure in SPSS 24 (IBM) with fixed-effects for the intercept, training, hemisphere (left or right), and contrast type [trained (reward or no-reward)] or [symbolic (reward—no-reward)], identity covariance structure, and maximum-likelihood estimation.

Whole Brain Analysis

Each subject's individual-level contrast from the GLM was normalized to the Labrador Retriever atlas space via the Advanced Normalization Tools (ANTs) software. Spatial transformations included a rigid-body mean EPI to structural image, affine structural to template, and diffeomorphic structural to template. These spatial transformations were concatenated and applied to individual contrasts from the GLM to compute group level statistics. 3dttest++, part of the AFNI suite, was used to compute a t-test across dogs against the null hypothesis that each voxel had a mean value of zero. All contrasts from the GLM mentioned above were included. The average smoothness of the residuals from each dog's time series regression model was calculated using AFNI's non-Gaussian spatial autocorrelation function 3dFWHMx –acf. The acf option leads to greatly reduced FPRs clustered around 5 percent across all voxelwise thresholds [52]. AFNI's 3dClustsim was then used to estimate the significance of cluster sizes across the whole brain after correcting for familywise error (FWE). Similar to human fMRI studies, a voxel threshold of $p \leq 0.005$ was used, and a cluster was considered significant if it exceeded the critical size estimated by 3dClustsim for a FWER ≤ 0.05, using two-sided thresholding and a nearest-neighbor of 1.

Combined Object Localizer and Affordance Analysis

For the 7 dogs that participated in both the object localizer study and the affordances study, we created a combined model that included the movies of the trained objects and the presentation of the trained objects. Preprocessing of the data was the same as described previously. Task related regressors for each experiment was modeled using AFNI's dmUBLOCK and stim_times_IM functions and were as follows: (1) 3Dmouth; (2) 3Dpaw; (3) 3Dneutral; (4) 2Dmouth; (5) 2Dpaw; (6) 2Dneutral. The first three contrasts for the combined model examined the differences in activation between the 3D and 2D versions of the affordance stimuli and were: [3Dmouth—2Dmouth], [3Dpaw—2Dpaw], and [3Dneutral—2Dneutral]. The contrast [affordance—neural] examined the difference between stimuli associated with affordances and those that were not, regardless of the objects' dimensionality. The contrast [all_3D—all_2D] was performed to test for perceived differences between all 3D and all 2D stimuli. The interaction contrast probed the difference between affordance and neutral stimuli modulated by object dimensionality [((3Dmouth+3Dpaw)—3Dneutral)—((2Dmouth+2Dpaw)—2Dneutral)].

RESULTS

Object Localizer Results

Individual Object Regions

Three dogs (Velcro, Rookie, and Zoey) failed to complete three runs of the object localizer task such that there was insufficient data to localize object-specific regions in the brain. Twelve dogs had object selective regions defined by the [all_objects—faces contrast] (for dogs that were not trained on affordances) or the [novel_objects—faces contrast] (for dogs trained on affordances) (Table 2). The contrast [objects—scrambled] was not used for the localizer as dogs frequently left the scanner or appeared to have aversion to the dynamic scrambled stimuli. No ROI was included within the frontal lobe of the canine brain, as comparable regions such as the LOC in humans are in the occipital and parietal lobes. The ROI for each dog was transformed to a Labrador retriever atlas (Fig 3).

Table 2. Object region size and threshold significance

Dog	Voxels (N)	T	*p*
Bhubo	26	2.178	0.03
Caylin	22	3.268	0.001
Daisy	37	2.91	0.01
Eddie	32	2.989	0.003
Koda	20	2.331	0.020
Libby	39	2.831	0.005
Ohana	32	2.31	0.022
Ollie	38	3.11	0.002
Pearl	39	2.8	0.006
Tallulah	26	2.06	0.04
Truffles	27	2.34	0.02
Zen	28	2.336	0.02

Dogs' Object ROI voxel size, t statistic, and p value for thresholds are listed

For the 7 dogs that participated in the object localizer study and the affordances study, we examined whether there was an effect of novelty on the neural activation to movies of objects within each dog's object region. We extracted beta values from each dog's object region for the contrast [novel objects—trained objects] and found that there was an effect of novelty, such that dogs had greater activation in object regions when viewing movies of novel objects relative to trained objects ($t(6) = 3.47$, $p = 0.013$).

Whole Brain Analysis

Whole brain analysis of the contrast [objects—faces] revealed significant activation for three clusters that survived a voxel threshold of 0.005 across dogs and a and cluster threshold of $p<0.05$. Regions included a medial lateral occipital region (845 voxels) and regions of the right occipitotemporal (393 voxels) and right parietotemporal lobe (279 voxels). These regions appear in the dog brain in analogous regions to the human brain for processing objects, including the lateral occipital complex (LOC) and anterior intraparietal sulcus (aIPS) (Fig 4).

Affordance Results

Object-Affordance Discrimination

On average, 10 dogs accurately performed the correct object-affordance behavior during the scanning session when allowed to interact with the paw object (M = 96%, SD =4%) and the mouth object (M = 87%, SD = 17%) (Fig 5). There was not a significant difference in the percentage of correct responses to the paw or mouth objects (*t* (16) = 1.56, *p* = 0.13). However, two dogs (Tallulah & Velcro) did not perform any affordance when presented with the paw object in the scanner, and Koda did not perform any affordances in the scanner because this resulted in too much movement during the remaining scan session.

Object Region Analyses

We examined the differences in percent signal change in response to the presentation of real-world objects in the object region of the dog cortex. There was not a significant difference between 3D objects associated with trained affordances and a familiar 3D object [affordances—neutral] (t(6) = -0.68, *p* = 0.52). However, there was a significant difference between the 3D objects associated with the paw affordance versus the mouth affordance [paw—mouth] (t(6) = 3.11, *p* = 0.02), such that dogs had greater neural activation in object regions for objects associated with a mouth affordance over a paw affordance (Fig 6).

Whole Brain Analyses

There were no regions identified in a group analysis across dogs that survived whole-brain correction for multiple comparisons.

Individual Affordance Regions

At the individual level, we found neural regions important for the differentiation of affordances associated with objects compared to neutral objects. Eleven dogs had left or right parietal regions selective for object stimuli associated with affordances. Each dog's affordance-specific region was defined by thresholding the statistical map for the contrast [affordances—neutral] until the number of voxels in each ROI was approximately 40 voxels, comparable to the size of the aIPS affordance regions defined in human fMRI studies (Table 3). The ROI for each dog was transformed to a Labrador retriever atlas (**Fig 7).**

Table 3. Affordance region size and threshold significance

Dog	Voxels (N)	T	p
Bhubo	23	2.011	0.045
Caylin	39	3.045	0.0024
Kaper	45	3.306	0.001
Koda	40	2.238	0.0258
Ollie	40	2.576	0.0102
Pearl	23	2.32	0.0208
Rookie	40	2.761	0.006
Tallulah	44	2.409	0.0163
Truffles	23	1.968	0.05
Velcro	35	2.45	0.0154
Zoey	40	2.291	0.0228

Dogs' Affordance ROI voxel size, t statistic, and p value for thresholds are listed

Dogs also had left or right parietal regions selective for object stimuli associated with the mouth affordance. Each dog's mouth-affordance region was defined by thresholding the statistical map for the contrast [mouth—neutral] until the number of voxels in each ROI was approximately 40 voxels (Table 4) (Fig 8).

Table 4. Mouth region size and threshold significance

Dog	Voxels (N)	T	p
Bhubo	24	1.966	0.5
Caylin	44	3.194	0.0015
Kaper	37	3.293	0.001
Koda	44	2.746	0.0063
Ollie	19	2.997	0.0028
Pearl	40	2.316	0.0211
Rookie	14	1.964	0.05

Tallulah	30	2.455	0.0143
Truffles	40	2.047	0.0415
Velcro	20	2.463	0.0141
Zoey	40	2.146	0.029

Dogs’ Mouth ROI voxel size, t statistic, and p value for thresholds are listed

Object Localizer Affordance Study ROI Analyses

For the 7 dogs that participated in the object localizer study and the affordances study, there were no significant differences for the contrast [novel objects—trained objects] in the regions from the defined from the affordance study. There was also no significant difference between the videos of objects associated with affordances and neutral objects [affordance-neutral] or between the two different affordances [paw-mouth].

2D to 3D Results

Caudate ROI Analyses

There was differentiation of the reward and no-reward stimuli in the caudate ROIs for the trained stimuli, regardless of whether dogs were trained on objects or pictures. In the caudate there was a significant interaction of training x [Reward – No Reward] (F (1,45) = 11.29, p = 0.002) (Fig 9).

Object Region Analyses

We examined the differences in percent signal change in the object regions defined previously for each dog in response to the presentation of 3D objects versus 2D versions of the objects. However, there were no statistically significant results for any of the contrasts in the object regions for the dogs.

Affordance Study ROI Analyses

For the 7 dogs that participated in the affordance study as well as the 2Dto3D study, we examined the differences in percent signal change for the contrasts in response to the presentation of 3D objects versus 2D objects associated with reward in the affordance regions defined previously. However, there were no significant differences in the affordance regions for any of the contrasts above.

Whole Brain Analyses

We found neural evidence for the differentiation of the reward and no reward stimuli as an effect of the dimensionality of the training stimuli. Whole brain analysis of the contrasts of interest revealed significant activation only for three contrasts that survive a voxel threshold of 0.005 (Table 5). The [trained reward—symbolic reward] contrast and the contrast comparing activation to the trained stimulus dimensionality versus the untrained stimulus dimensionality [trained reward + trained no-reward)—(symbolic reward + symbolic no-reward)] revealed a region in the posterior parietal lobe with greater activation toward the trained dimensionality of stimuli than the untrained dimensionality (Fig 10A). The contrast comparing the reward associations for the untrained dimension of stimuli [symbolic reward— symbolic no-reward] produced a region in the right anterior parietotemporal cortex (Fig 10B).

Table 5. Cluster size and threshold significance for 2D and 3D object processing regions

2D3D Contrast	**Region**	**Voxel threshold**	**Cluster Size (voxels)**
trained reward – symbolic reward	Left posterior parietal	.005	454 (p<.02)
symbolic reward— symbolic no-reward	Right parietotemporal	.005	248 (p=.05)
(trained reward + trained no-reward)—(symbolic reward + symbolic no-reward)	Left posterior parietal	.005	528 (p<0.01)

Object Localizer Dimensionality Region Analyses

For the 12 dogs who participated in the object localizer study, we examined whether regions defined from the 2D to 3D study were selective for objects. In one-sided t-tests, we found a significant difference in activation for the contrast [objects—faces] in the left posterior parietal region selective for stimulus dimensionality (t(11) = 1.831, p = .047) and for trained reward versus no reward (t(11) = 1.872, p = .044). There was no significant difference between objects and faces in the right parieto-temporal region selective for the untrained dimension of stimuli (t(11) = 0.946, p = .182).

For the 7 dogs that participated in the object localizer study and the affordances study, there were no significant differences for the contrast [novel objects—trained objects] in the regions defined from the 2D to 3D study.

Affordance Study Dimensionality Region Analyses

Using the left parietal region sensitive to the trained dimensionality of the stimuli [all trained—all symbolic] as well as the right parietal region sensitive to the untrained dimensionality of stimuli [symbolic (reward—no reward)] from the 2D to 3D study, we extracted beta values from group contrasts in the affordances study. A one-tailed t test was used to test for significant differences because the assumption was that there would be a greater activation for the affordance object than a neural object. There was a significant difference between objects associated with the mouth affordance versus no affordance [mouth-neutral] (t(10) = 2.1528, p = 0.05) in the right parietal region (defined from the [symbolic reward—symbolic no-reward] contrast). There was also a significant difference between the objects associated with affordances versus nothing [affordances—neutral] (M = 0.32, SD = .49) (t(10) = 2.17, p = 0.05) in the left parietal region.

Combined Object Localizer and Affordance Analysis Results

Object Region Analyses

There were no significant differences for the affordance contrasts in individual dogs' object regions.

Dimensionality Region Analyses

Using the left parietal region sensitive to the trained dimensionality of the stimuli [all trained—all symbolic] from the 2D to 3D study, we extracted beta values from group contrasts in the combined localizer affordances studies model. The contrast [all_3D—all_2D] was significant for 6 dogs in this region, indicating that this region is most sensitive to 2D stimuli associated with affordances ($t(5) = -2.85$, $p = 0.036$).

DISCUSSION

Our fMRI results provide the first evidence for dedicated regions in the occipital and parietal cortices for the processing of objects in dogs. The main finding is that dogs' perception of objects is influenced by the possible affordances the dog associates with the object. We then examined how different types of affordances affect neural activity with object regions of the dog brain. In these object regions, the objects associated with a mouth affordance elicited stronger changes in the BOLD response than the objects associated with a paw affordance. Further, whole-brain analyses revealed discrete regions for processing general affordances in an anterior parietal region and prioritization for the mouth affordance in the parietal cortex. While there was variability across dogs in the locations of the object or affordance regions, there was marked overlap between dogs, suggesting that these regions are functionally consistent across dogs. We also searched for effects of stimulus dimensionality in object regions for the processing of 2D and 3D versions of objects. Although there was no difference in BOLD activation based on stimulus dimensionality in object regions, whole-brain analyses revealed a left posterior parietal region selective for the trained dimension of stimuli over the untrained dimension. Taken together, these findings suggest that dogs' neural representation of objects depends on the object's actionability and dogs' familiarity with object dimensionality. In sum, dogs may perceive object stimuli with the initial question of "Can I put this in my mouth?"

Object Regions

The results for the object localizer were consistent with those of human fMRI studies. We were able to localize regions of visual cortex selective for objects in each dog using the same videos of object and face stimuli as those used in human fMRI studies. Across individual dogs, we found that these object regions overlapped in either the left or right hemisphere of the occipital lobe. Despite differences in brain morphology across the breed and size of dogs, our results suggest that dogs share a lateral occipital region selective for viewing object stimuli.

In humans, viewing real objects as well as images of objects activates similar networks, particularly the lateral occipital complex along the lateral and ventral convexity of occipito-temporal cortex [36, 53]. However, in a human fMRI study that presented real objects and pictures of the objects, the LOC in particular was sensitive to visual differences between the two, such that LOC does not code the real (3D) and pictorial (2D) versions of a shape as equivalent (Snow et al., 2011). Because real objects afford specific actions, including the graspability of an object or if it is within reach of the dominant hand, object-specific affordances may have a unique effect on neural responses to the different versions of the same object stimuli [37, 39, 54, 55]. Unlike humans, we found little difference in dogs' neural activation in individual object regions between 2D and 3D versions of object stimuli associated with reward. Our finding of similar neural activation in object regions of the dogs' brains to 2D and 3D versions of object stimuli could be due to dogs forming an abstract object concept that is invariant to the dimensionality of the object. However, as the object-reward pairings were acquired using a passive viewing task, dogs had little experience interacting with the objects or picture versions of the objects. A more likely explanation for our finding is that the study was insufficiently powered given the number of dogs or that dogs' lack of affordance-associations with either object may therefore have made both objects and pictures of objects equivalent to the dog as neither was actionable.

To test this explanation, we trained dogs on specific object-affordance pairings and examined whether affordances affected BOLD activation within object-selective regions of the dog brain. We found that individual object regions responded more to videos of novel objects than to videos of familiar objects, consistent with previous dog fMRI studies that show increased neural activation to novel objects or novel words [43, 44]. Further, dogs' apparent preference for the mouth affordance also influenced brain activation within individual object regions: there was a greater neural response to real objects associated with the mouth affordance than to objects associated with the paw affordance. This contrasts with human fMRI studies, which show differences in neural activation based on affordances within parietal regions, but not in primary visual or object regions [41, 56]. As learned stimulus-reward associations can have feedback-related effects on primary sensory areas, it is possible that dogs' increased activation to mouth affordances in individual object processing regions reflect how preferences for mouth affordances may bias dogs' visual perception of objects [57].

Affordance Regions

We found regions in parietal cortex of each dog that selectively responded to real objects associated with affordances. Across individual dogs, we found that these affordance regions overlapped in either the left or right hemisphere of the parietal lobe. These overlapping affordance regions are similar to brain regions found in primates associated with processing object-related affordances. In nonhuman primate studies, the core regions involved in grasping include the anterior intraparietal area (AIP) and premotor area f5 [58]. In human studies, the anterior intraparietal sulcus (aIPS) and ventral premotor cortex (PMv) are activated during grasping and show selectivity for grip type. The aIPS in particular shows differential activation based on the realness of the object (either 3D or 2D) and the motor task performed on the object (e.g. grasping or reaching) [39, 41]. In dogs, we not only found similar parietal regions to the aIPS selective for affordances, but also overlapping regions across dogs in the anterior parietal lobe that were selective for the mouth affordance. Like humans, dogs' BOLD response in affordance areas increased during the presentation of the affordance objects (e.g. the planning phase) before any initiation of movement. These differences in activation were mirrored across species, as humans' aIPS activation was greater for grasping movements (i.e. dogs' mouth affordance) than for reaching movements (i.e. dogs' paw affordance). In humans, neural biases in the aIPS towards grasping affordances are likely due to fine-grained planning and anticipation of the consequence of the interaction with the 3D object, which also could be an explanation for dogs' neural biases for the mouth affordance [41]. Human fMRI studies have also shown that reward-based associations between visual stimuli and outcomes alters activation in the middle frontal gyrus, superior frontal sulcus, posterior cingulate cortex, and the inferior parietal sulcus [57]. So, dogs' greater activation for the mouth affordance in parietal regions could also be explained by the hedonic value associated with an affordance. Additionally, dogs also may prefer the mouth affordance to perform grasping simply because they lack opposable thumbs.

Unlike human imaging studies, we did not find a significant difference in affordance regions between the video and real versions of objects associated with affordances. In human fMRI studies, actions are affected by the realness of the object stimuli even when planning how to interact with the object [39]. The left aIPS is thought to be selective to object realness to generate forward models of planned actions that account for the action's constraints and outcomes, such as proprioceptive feedback, that are associated more with real objects than with images [41, 56]. Objects and pictures of the same objects elicit different patterns of fMRI adaptation in the dorsal pathway of object recognition, potentially reflecting differences between objects and images in the affordances that are available for each [36]. As the BOLD response in dogs' parietal affordance regions did not differ between the real and video versions of objects associated with affordances, a possibility is that the dorsal-stream object representations included the potential actions associated with objects, which were also evoked during when viewing movies [41, 59, 60]. However, that is not to say that dogs used the videos of objects as symbolic representations for real objects. Indeed, behavioral studies show that dogs rarely generalize behavior to pictures from the real world objects they represent without substantial training [14, 61]. This, and the low number of dogs that completed both the affordance and 2D to 3D study, suggests that dogs' spontaneous generalization of affordances from real world objects to movies of the same objects is unlikely.

Dimensionality Regions

As most studies of canine cognition rely on visual stimuli, we examined whether dogs use hedonic neural mechanisms to generalize from pictures of objects to real world objects. In the interaction contrast, we found that dogs show greater activation within the caudate nucleus to the trained dimension of stimuli relative to the untrained dimension (e.g. dogs trained on pictures of objects had greater activation to pictures relative to real world objects), suggesting hedonic neural mechanisms are biased toward the dimensionality of stimuli with which they are more familiar. Additional brain regions selective for stimulus dimensionality included a left posterior parietal region across dogs where there was greater activation to the trained dimensionality of stimuli than to the untrained dimensionality, which appeared in the same region but opposite hemisphere as the LOC defined in the object localizer study (Fig 11). Multi-voxel pattern analysis (MVPA) of human imaging data supports these findings, as patterns in object regions can be different for object exemplars from the same category that vary based on viewpoint or size, as well as between 2D and 3D versions of the same objects [32, 36]. Consistent with human imaging studies, the left posterior region also showed greater activation to objects relative to faces across dogs and appeared in regions of the canine brain similar to the primate lateral occipital complex (LOC) [41, 56].

There was also greater activation to the untrained reward versus no reward stimuli in a right parietotemporal region across dogs (e.g. dogs trained that the 2D giraffe was the reward stimulus had greater activation to the 3D giraffe than the 3D whale in this region). Greater activation to the untrained reward stimulus in this region provides some evidence that dogs use hedonic neural mechanisms to generalize a stimulus-reward association from the trained reward stimulus to the untrained stimulus. However, we do not know what features, such as color or shape, that the dog may use to facilitate this representation. There was also greater activation in this same region to the video versions of affordance-object stimuli than to the real-world affordance objects from the dogs' training. In human fMRI, the right primary visual cortex (V1) and right inferior temporal gyrus also showed greater activation to 2D versions of objects versus 3D objects [36]. Additionally, there was a significantly greater BOLD response to objects associated with affordances relative to neutral objects in this region and greater activation for the mouth affordance object versus the neutral object. Together, our results suggest that like humans, dorsal regions of the dog brain may process abstract features of object stimuli that include, but are not limited to, affordances [56].

There were several limitations to our studies, the foremost being that we had a low number of dogs participate in the affordances or localizer study. Dogs or their owners were unavailable to train the affordance behaviors during the experiment and some dogs were unable to remain still while viewing video stimuli in the MRI. Further, we limited the number of objects to two or three items per study, which, while creating a simple controlled design with many trials per item, may limit the generalizability of our findings to all objects that a dog may encounter. Unlike human imaging studies, we also did not include more abstract object stimuli that were composed only of lines or were limited in color to black and white. Because dogs have a high flicker fusion rate and a visual streak, and are therefore more likely to attend to movement of objects across the periphery, we used only movie stimuli for the object localizer as opposed to static images. We were also unable to use the data from the objects-scrambled contrast for the localization of object regions as is common in human fMRI. Most of the data removed during preprocessing of the localizer data was due to motion during the presentation of the scrambled object movies, which may have been aversive to the dogs. Lastly, as the object localizer was conducted on the same day as the affordance study, we limited the object localizer to 3 runs. This limited number of trials within the localizer such that the leave-one-out analysis to confirm the object regions' selectivity was underpowered. To address these concerns, future research could confirm the selectivity of object regions for each dog using novel stimuli.

Conclusions

Our fMRI results provide the first evidence for dedicated regions in the occipital and parietal cortices for the processing of objects in dogs. The main finding is that dogs' perception of objects is influenced by the possible affordances the dog associates with the object. Although real objects and pictures of the same objects share a degree of visual similarity, they differ fundamentally in the affordances associated with them. This is especially true for dogs, as they can manipulate objects only with their mouths or paws. Dogs' object affordances are thus most like infants, who initially interact with the world by placing everything within reach directly into their mouths. Like humans, we have begun to understand how dogs perceive their world through brain imaging, as this offers direct insight from the participant about the neural mechanisms underlying perception. Rather, our studies reveal that there are potentially shared neural mechanisms underlying dogs' and humans' visual perception of objects, and that neural biases may in turn affect perception and behavior. Together, ours and other studies provide insight into the question of whether pictures are an appropriate proxy for real world stimuli for dogs and for fMRI.

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Figure 1. Object Affordance Training. Left) Dog Rookie demonstrating mouth affordance on die object. **Right)** Dog Tallulah demonstrating paw affordance on die object.

3D Objects 2D Pictures of Objects

Figure2. 2D to 3D Stimuli. Left) 3D whale and 3D giraffe objects attached to 2.5-foot dowels for presentation of stimuli to dogs while in the scanner. **Right)** Pictures of the whale and giraffe 3D objects were printed to create 2D color-matched versions of the 3D stimuli and pasted to foam board and 2.5-foot dowels for presentation of 2D stimuli to dogs while in the scanner.

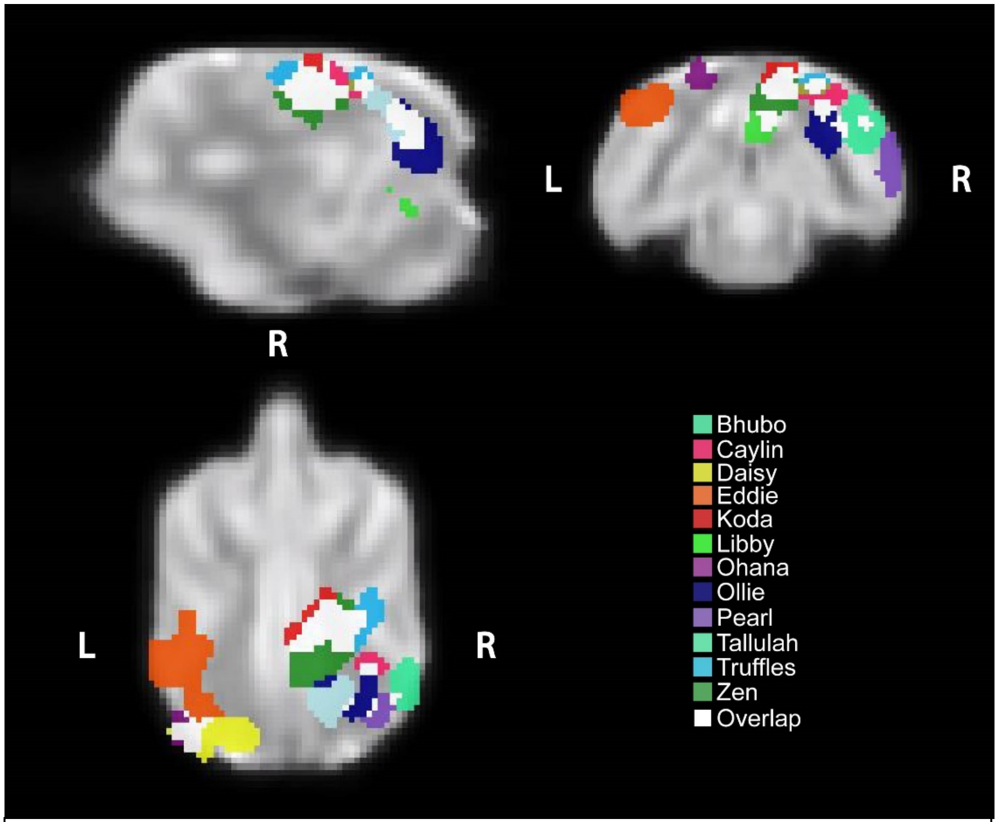

Figure 3. Individual Dog Object Regions. Sagittal, transverse, and dorsal sections. Regions were defined using the objects-faces contrast of video stimuli for each dog. Colors represent individual dogs; white represents overlap between two or more dogs.

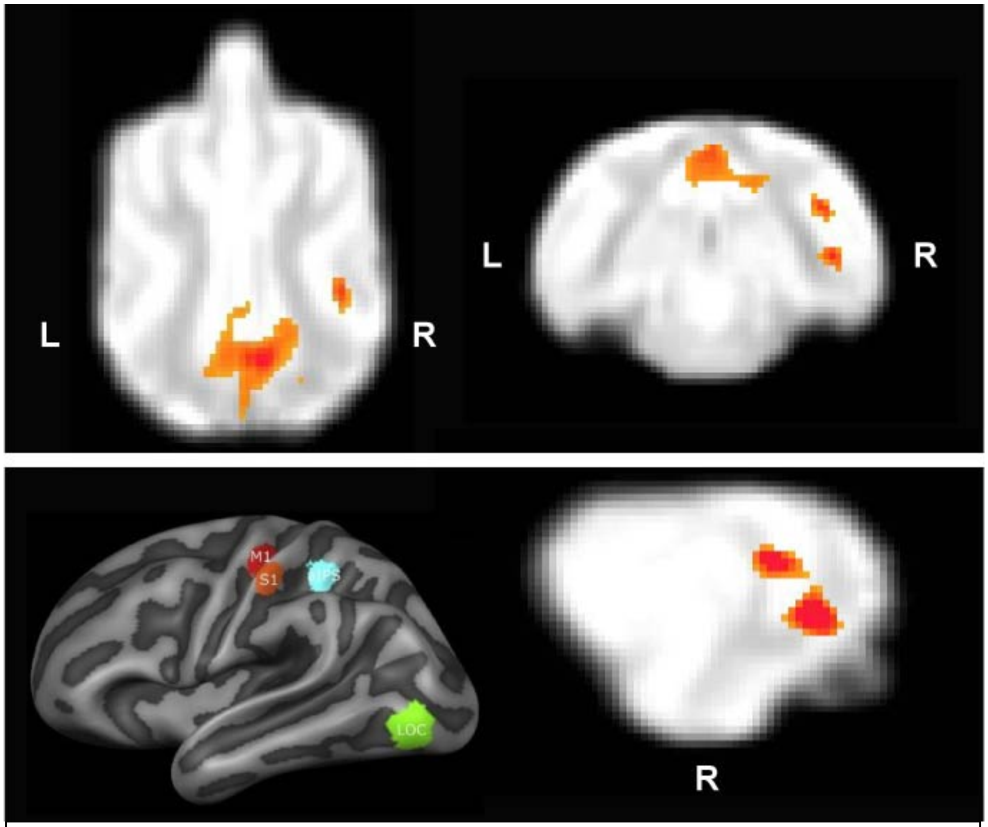

Figure 4. Group object processing region for object stimuli > face stimuli. Whole brain analysis of the contrasts of interest revealed significant activation for three clusters that survive a voxel threshold of 0.005. **Top) Dorsal and transverse sections of the dog brain.** Regions included a medial occipital region (845 voxels) and regions of the right occipitotemporal (393 voxels) and right parietotemporal lobe (279 voxels) **Bottom) Comparison of sagittal sections of the human (left) and dog (right) object processing regions.** Contrasts from the present study show activation in the dog brain in analogous regions to the human brain for processing objects, including the lateral occipital complex (LOC) and anterior intraparietal sulcus (aIPS) [1].

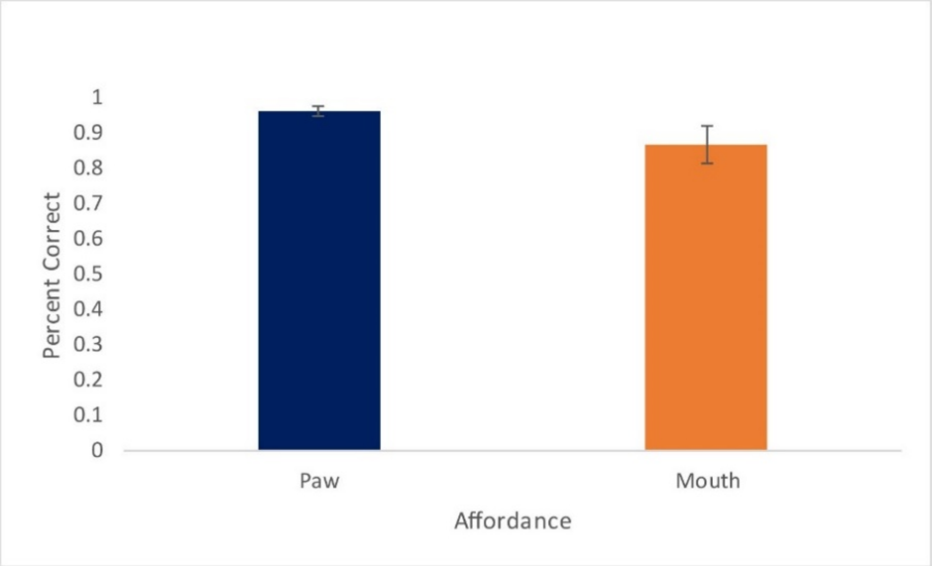

Figure 5. Dog's behavioral performance on affordance blocks. 10 dogs accurately performed the correct object-affordance behavior during the scanning session when allowed to interact with the paw object and the mouth object. There was not a significant difference in the percentage of correct responses to the paw or mouth objects (*t* (16) = 1.56, *p* = 0.13). Two dogs (Tallulah & Velcro) did not perform any affordance when presented with the paw object in the scanner.

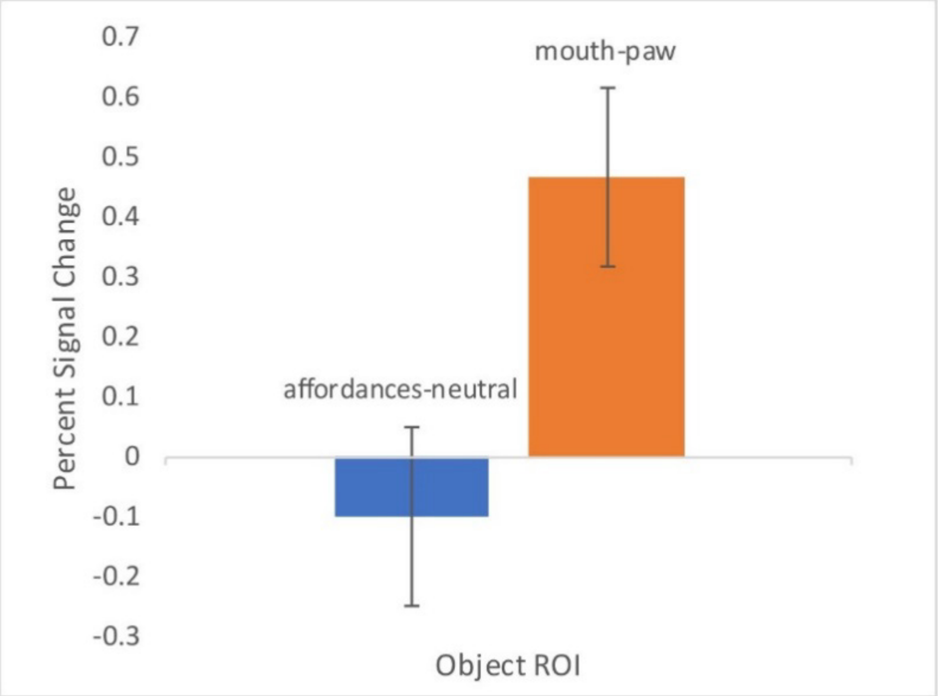

Figure 6. Beta values (Percent signal change) in individual dogs' object regions for contrasts of interest in Affordances study. We examined the differences in percent signal change in response to the presentation of real-world objects in the object region of the dog cortex. There was not a significant difference between objects associated with trained affordances and familiar objects (t(6) = -0.68, *p* = 0.52). However, there was a significant difference between the objects associated with the mouth affordance and the paw affordance (t(6) = 3.11, *p* = 0.02).

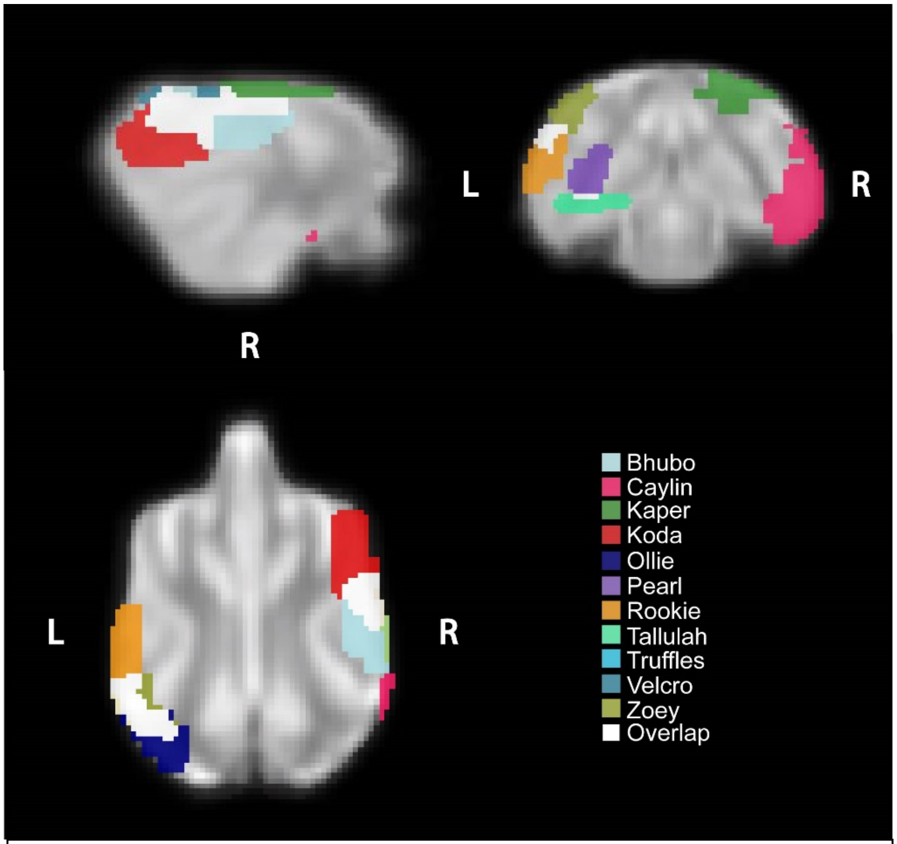

Figure 7. Individual brain regions for processing object stimuli associated with affordances. Sagittal, transverse, and dorsal sections. We found neural regions important for the differentiation objects associated with affordances relative to neutral objects that were familiar but associated with nothing. Colors represent individual dogs, white represents overlap between two or more dogs.

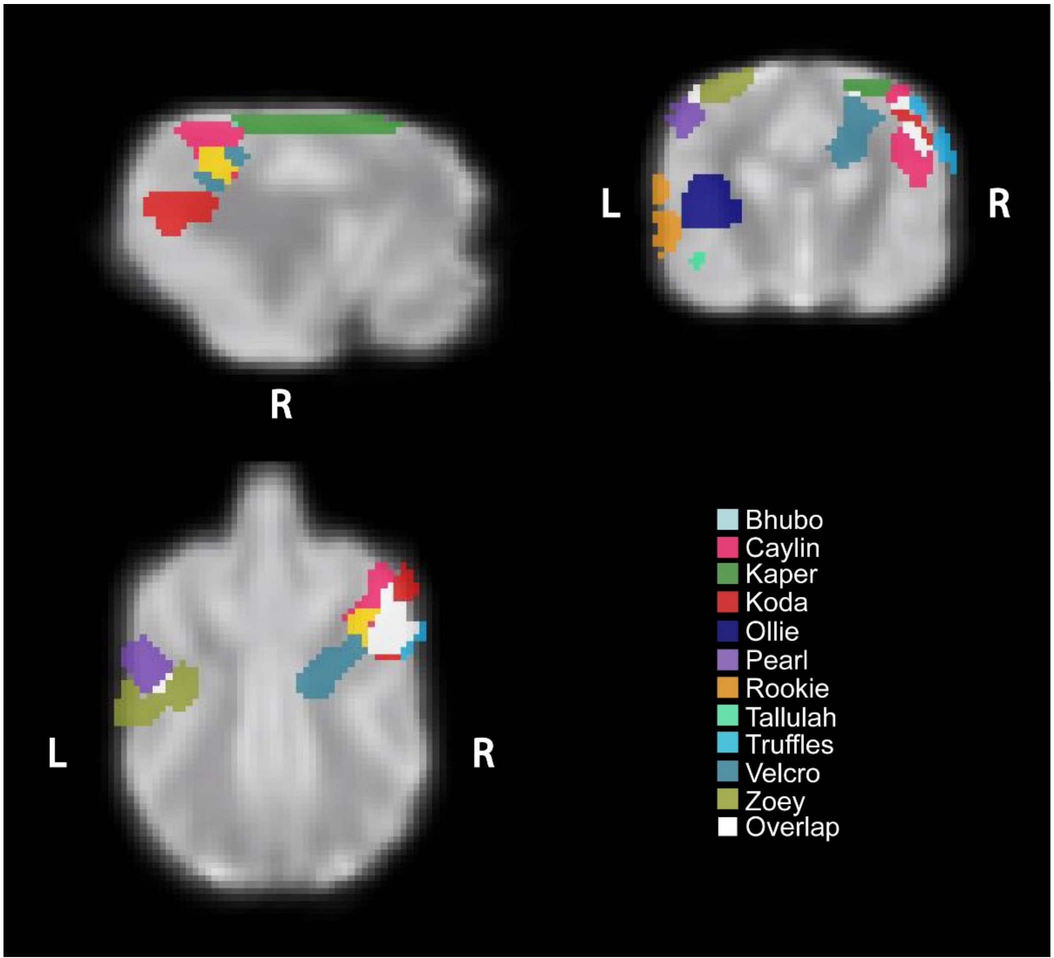

Figure 8. Individual brain regions for processing object stimuli associated with mouth affordances. Sagittal, transverse, and dorsal sections. We found neural regions in each dog that showed greater activation to the object associated to the mouth affordance relative to the neutral object. Colors represent individual dogs, white represents overlap between two or more dogs.

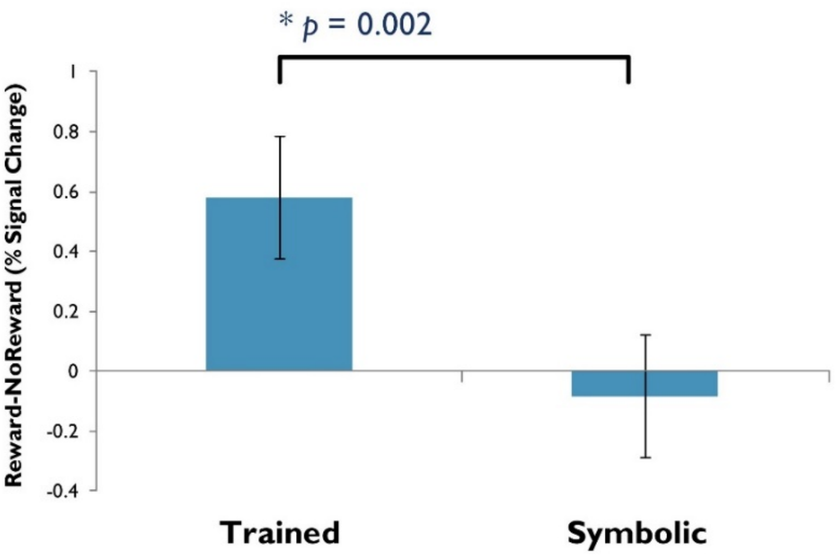

Figure 9. Average beta values (Percent signal change) in individual dogs' caudate nucleus for the contrast of Reward—No Reward separated by training and testing (symbolic) stimuli. Changes in brain activation were extracted from contrasts in the 2D to 3D study. In the caudate there was a significant interaction of training x [Reward – No Reward] (F (1,45) = 11.29, p = 0.002).

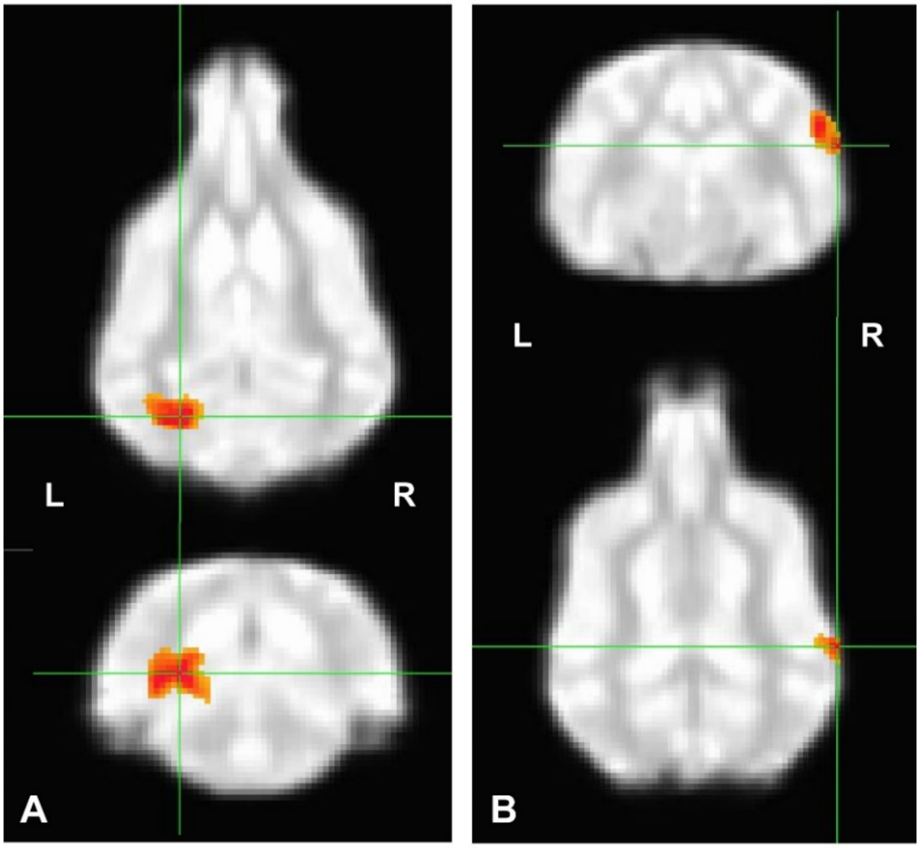

Figure 10. Regions important for the discrimination of dimensional object stimuli. Whole brain analysis of the contrasts of interest revealed significant activation only for three contrasts that survive a voxel threshold of 0.005. **A) Dorsal and transverse sections.** The [trained reward—symbolic reward] (454 voxels) contrast and the contrast comparing activation to the trained stimulus dimensionality versus the untrained stimulus dimensionality [trained (reward + no-reward)—symbolic (reward + no-reward)] (528 voxels) revealed a region in the left posterior parietal lobe with greater activation toward the trained dimensionality of stimuli. **B) Transverse and dorsal sections.** The contrast comparing the untrained dimension of stimuli [symbolic reward— no-reward] (248 voxels) produced a region in the right anterior parietotemporal cortex.

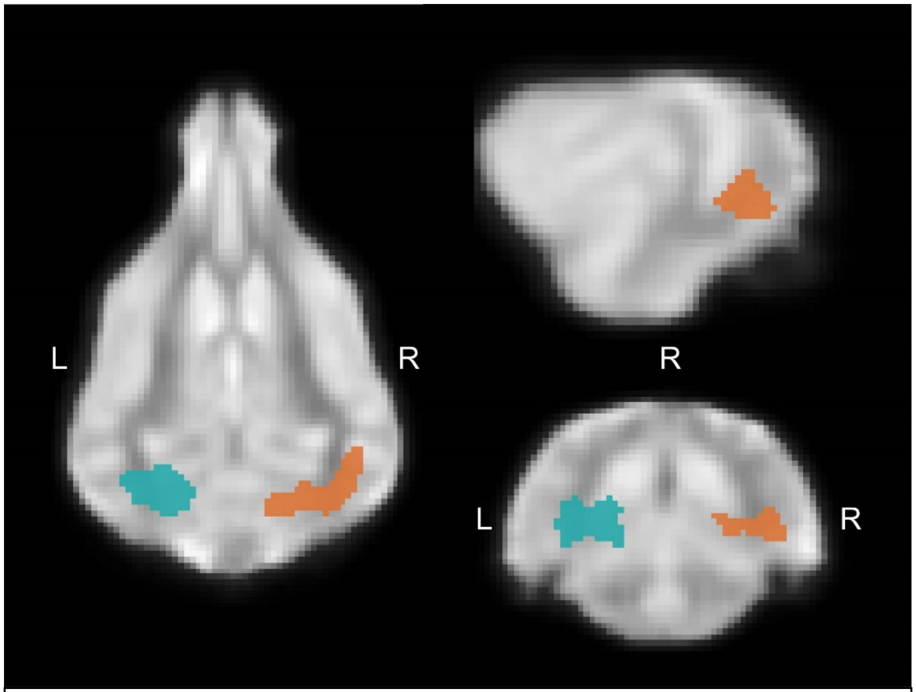

Figure 11. Group LOC across studies. Dorsal, sagittal, and transverse sections comparing bilateral regions for processing object stimuli. **Orange** designates the region (393 voxels) selective for object stimuli from the object localizer study. **Blue** designates the region (528 voxels) selective for the trained stimulus dimensionality versus the untrained stimulus dimensionality from the 2D to 3D study.

GENERAL DISCUSSION

The results of this dissertation inform our understanding of the neural mechanisms underlying dogs' perception of their environment. The first manuscript compares the rates of associative reward-learning in the brain across visual, olfactory, and verbal modalities. The studies show that dogs have neural mechanisms that support a bias for learning visual and olfactory stimuli at a faster rate than verbal stimuli. After establishing that dogs have neural biases for specific modalities of stimuli, the dissertation follows with two empirical papers to better understand how dogs process stimuli within a modality. The second paper provides neural evidence for auditory novelty detection in the domain of human speech. This study shows that dogs process human speech at least to the extent of differentiating words they are familiar with from new words. But beyond novelty detection, dogs also may share analogous regions to humans for differentiating between known words, though further study is needed to bear this out. The third paper focuses on dogs' perception within the visual domain, specifically whether dogs' perception of objects is affected by a) the possible affordances dogs' associate with an object and b) whether the object is presented as a 2D or 3D version. The studies indicate that dogs' have a neural bias for objects that they can put in their mouth as well as differences in their processing of dimensional stimuli based on their relative familiarity with either dimension. Together, the three papers describe a pattern of neural biases in dogs that are likely due to physiological and environmental factors that, when combined with a general preference for novelty, influence perceptual biases. Below, the main findings and their potential implications are discussed.

Neural biases for sensory modalities

In a series of three experiments, we demonstrated the use of fMRI in dogs to compare associative reward-learning in the brain across visual, olfactory, and verbal modalities. There were significant differences in the time courses, suggesting that although multiple modalities are represented in reward-processing regions of the canine brain, the rates of acquisition and habituation are modality-dependent. In this manuscript we show that dogs acquired the reward associations with odors and visual stimuli at a faster time course than verbal stimuli.

The effects of stimulus modality on differential neural time courses highlight the potential implications for training dogs. Most training protocols for dogs use gestural and verbal commands. While optimal for humans, these protocols may not be the most effective for learning from a dog's perspective. When olfactory information is present, the dog may attend to olfactory sensory information over visual information and will likely attend to both domains over verbal information. Although dogs attend to verbal stimuli, neural biases for olfactory and visual stimuli likely alter the dog's awareness of its physical and social environment. Our results, showing greater salience for olfactory and visual stimuli, are in line with, and may potentially contribute to, dogs' behavioral preferences in their natural surroundings.

Neural Mechanisms for language processing

Given that most humans communicate with dogs using verbal commands, we used fMRI to identify neural mechanisms of dogs' processing of human words. We found neural activation was greater for novel pseudowords relative to trained words. The mechanism of such novelty detection may be rooted in either the relatively less frequent presentation of the pseudowords (oddball detection) or the lack of meaning associated with them (lexical processing). This leaves the question of what the words represent to dogs? One possibility is that the words had no further representation other than the relative hedonic value of the objects, given that activation was present in reward-processing brain regions. However, the additional regions identified, such as the TPJ, appear in analogous regions to those in the human brain for processing the semantic content of words. While this manuscripts' results imply that these regions may also process words or even semantic representation in dogs, further work is needed to verify the functions of these regions in the dog brain.

Neural regions for processing objects

In three experiments, we provide evidence for dedicated regions of the dog brain for processing objects. Although real objects and pictures of the same objects share a degree of visual similarity, they differ fundamentally in the affordances affiliated with them. We found that like young children, dogs have a perceptual bias for objects based on their ability to manipulate the object with their mouth[167]. We then examined how different types of affordances affect neural activity, which revealed discrete regions for processing general affordances in regions analogous to those in the human brain. We also demonstrated that dogs' experience with stimulus dimensionality alters activation in brain regions based on the dog's familiarity with 2D and 3D versions of objects. As our study directly examined dogs' perception of real-world objects and pictures, our findings provide novel evidence that dogs' perception of objects is affected by both the realness of the visual stimulus as well as dogs' ability to interact with the object with their mouth. Our studies also reveal that there are potentially shared neural mechanisms underlying dogs' and humans' visual perception of objects. Together, ours and other studies provide insight into the question of whether pictures are an appropriate proxy for real world stimuli for dogs and for fMRI.

Limitations of dog fMRI

There are several potential limitations across the three manuscripts. First, although we isolated the salient modality in separate experiments, the presence of the human owner was generally constant throughout the experiments. Because the human was not blind to the nature of the stimuli, they could have inadvertently influenced the associative process through body language. However, because the olfactory stimuli were least likely to be picked up by the humans and were not saliently communicated by human owners, as were the display of the visual objects or the vocalization of the auditory stimulus, so-called 'Clever Hans' effects are unlikely to explain our results.

Second, some statistical analyses were underpowered due to sample sizes across studies. In the first manuscript, we examined changes in neural activation across regions of interest and across dimensionality in 19 dogs. As dogs' participation in this series of three studies required no additional training prior to scanning, many dogs were available to participate. In the second manuscript, owners were required to train their dogs for approximately four months on a word-object discrimination paradigm. Because of the time commitment and training required for this study, only 11 dogs were available to participate. In the third manuscript, two of the studies required no additional training but took place one year apart, such that more dogs were available to scan for the dimensionality study than for the object localizer study. The localizer study also required that the dogs would maintain a down-stay while watching videos, and not all dogs were comfortable with their owner being completely out of view or with watching movies. In the affordance study, owners were required to train their dogs on an object-affordance paradigm, which while requiring approximately one month to train, reduced the number of dogs available to participate in the study. Given that some of the studies were potentially underpowered, a non-significant effect does not mean that the effect does not exist.

Fourth, the effects of habituation counteract those of learning. There is ample evidence that brain structures like the amygdala habituates to repeated presentations of the same stimuli. It would not be surprising that repeated presentation of the stimuli or repeated fMRI scans could lead to dogs' decreased physiological response. Most dogs included in the manuscripts also had experience from previous fMRI studies with conditioned object-reward or odor-reward associations, with picture and movie stimuli, and with a fake dog, such that particular stimuli within the scanner environment may have been more novel than others.

Finally, the manuscripts included studies where conditioned associations were acquired either through a passive task in the scanner or through behavioral training outside of the scanner. In the first manuscript and the dimensionality study, stimulus-reward associations were acquired through a passive task in the scanner. In the second manuscript and the affordance study, behavioral tests of the dogs' ability to discriminate between word-object associations or affordance-object associations were conducted prior to scanning. In contrast, no behavioral tests were conducted to test acquisition of the learned associations or to compare to the neural activations in the first manuscript. Thus, while we measured behavioral performance for some of the studies, we do not have enough to infer a directional relationship between neural mechanisms and behavioral outcomes across all studies.

Implications

Like humans, we have begun to understand how dogs perceive their world through brain imaging, as this offers direct insight from the participant about the neural mechanisms underlying perception. Together, these manuscripts add to our understanding of dogs' perception by assessing changes in neural activation when dogs were presented with sensory stimuli and identifying brain regions that processed the incoming sensory information. We also extend the literature by moving beyond behavioral measures to ask how dogs' neural responses are modulated based on the modality or dimensionality of the stimulus.

More broadly, our data speak to the perceptual differences between humans and dogs, and how these can inform our future interactions with dogs. As we show that dogs may attend to certain modalities –notably visual and olfactory—more than verbal stimuli, this suggests that humans' propensity to talk to their pets is based on humans' perceptual preferences rather than the dog's innate aptitude. Practically, our results can inform how we train dogs in the future and possibly decrease the time it takes for the dog to learn something new. For example, when training a dog, humans should use a combination of gestural and verbal cues to issue a command. The gesture is most salient to the dog when they are near the owner, whereas a verbal cue would be necessary to instruct the dog when the owner is out of the dog's line of sight.

While our results suggest that dogs have some aptitude for differentiating between words, this seems to be highly based on their familiarity with what the person says, such that the dog's behavior is shaped by hearing words or a tone that sounds like what they have heard previously. Though we, as dog owners, would like to think that dogs understand our speech, they do at least seem to process familiar words, and may be trying to match new words to the ones that they are already familiar with. To a human, this may appear that the dog is slow on the uptake or unable to learn something new, but in actuality, a form of language processing may be taking place in the dog's mind.

Lastly, we are constantly encountering new technologies that use digital media for communication, and dogs seem to be no exception. Dog owners provide access to dog-directed television or applications for their dogs because they believe their dogs attend to the content appearing on the screens of cell phones, iPads, and televisions. Our results suggest that what dogs get out of these forms of stimuli varies based on their experience with 2D stimuli, and if they perceive the stimulus as something they can put in their mouth. These data shed light on not only perceptual features of the dog-human bond, but the how future interactions may be mediated as an effect of technology. Additional work on understanding dogs' perception is clearly warranted to inform our relationship with dogs as well as create mutually beneficial situations for both humans and dogs.