Investigating the Dog-Human Social Bond via Behavioral and fMRI Methodologies

by

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Abstract

Domestic dogs have been bred into a close relationship with humans, and it is one in which they are often considered family members just as human children are. Given the development of this intense bond, it is unsurprising that dogs have developed a keen ability to interact with humans in an effective way. This ability to interact with humans has further led to dogs working alongside humans in tasks ranging from fetching a toy to sniffing materials in search of bomb components. Given that domestic dogs hold this position as an integral part of daily human life, it is important that we seek to understand canine cognition and behavior and how it is similar to and different from that of humans. In the current research, the dog-human relationship was investigated using a multi-method approach, applying established behavioral methods alongside neuroimaging techniques. The outcome of this research is a comprehensive assessment of individual and global behavioral and neural markers of social attachment in domestic dogs. First, the foundational literature for the research is detailed, including discussions of dog domestication and working dog proficiency. Next, a methodology is described which assesses neural indicators of face and emotion processing and recognition in dogs, as well as behavioral indicators of dogs' attachment to familiar humans. It was found that dogs demonstrated differential brain activations according to familiarity and emotional valence in the hippocampus, amygdala, and caudate and that such activations correlated with behavioral markers of attachment.

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List of Abbreviations

MRI	magnetic resonance imaging
fMRI	functional magnetic resonance imaging
C-BARQ	Canine Behavioral Assessment and Research Questionnaire
FFA	Fusiform Face Area
IAPS	International Affective Picture System
ITI	inter-trial interval
DSLR	digital single-lens reflex
JPEG	Joint Photographic Experts Group
AVI	Audio Video Interleave
IRB	Institutional Review Board
DARPA	Defense Advanced Research Projects Agency
T	Tesla
ISI	inter-stimulus interval
TR	Repetition Time
SPM	Statistical Parametric Mapping
SPSS	Statistical Package for the Social Sciences

Chapter 1: Introduction

Dogs and humans share a unique history that spans at least 18,000 years (Thalmann et al., 2013). It is undeniable that in those societies fostering the domestication of pet and working dogs, dogs have reached a unique social status unknown to any other non-human animal. There are numerous hypotheses for the origins of the dog and the role that humans played in its domestication. However, most of these are built on the foundation that grey wolves and humans began interacting in prosocial ways as humans settled permanently in villages, creating waste piles that provided sustenance for hungry wolves. The wolves that happened to be slightly less afraid of humans were more likely to find food, and thus a cycle began perpetuating in which lower levels of fear and aggression brought humans and canids closer and closer together. Through this domestication process, humans and dogs developed a complex social repertoire. Shettleworth (2010) defines social cognition as a conglomeration of all of the processes needed to know and act on information in the social environment, be them relevant to oneself or one's relationships with others. The utility of social skills is found in the ability to differentiate roles and predict behavior in order to maintain a stable environment. Needless to say, the deep and sustained bond between man and dog is exemplary of keenly tuned social cognition on the part of both species.

Topics and Progression

For the reader, I have supplied section headings throughout this dissertation so that a selective reading can take place. This section contains an outline of topics through which the dissertation progresses.

I first present a literature review of domestic dog domestication and social intelligence, which includes the origination and findings of the unsolvable task used in Experiment 2. Next, I review the development and findings of functional Magnetic Resonance Imaging (fMRI) with dogs, as well as provide a background of face processing studies. Finally, I discuss the current state of the working dog field as a foundation for the studies discussed herein. Following the literature review, I introduce and expand upon Experiment 1, which included visual stimulus development and the fMRI task, and Experiment 2, which targeted working behavior using the unsolvable task.

Hypotheses of Canine Social Intelligence

Reid (2009) describes four common hypotheses regarding social cognition in the domestic dog. The first postulates that through interaction with humans, dogs have learned to be responsive to their social cues on the basis of simple conditioning processes. The second hypothesis enlists the process of domestication, supposing that in addition to decreasing their fear of humans, dogs naturally applied general problem-solving abilities to their interactions with humans. The third focuses on cognitive mechanisms and suggests that the co-evolution of dogs and humans has not only enabled dogs to respond to our social cues, but also to understand our mental states. Finally, the fourth hypothesis comes from an ontological perspective and suggests that the abilities we see in dogs are learned rapidly during development in accord with a predisposition for such learning.

Viranyi and Range (2014) build on a hypothesis of domestication through fear reduction by noting that wolves possess preconditioned tolerance and social capabilities for managing intraspecific social relationships. Thus, such social capacities as attentiveness and tolerance may have transitioned easily to humans with a corresponding reduction in fear. Interestingly, these social capacities in domestic dogs have become heterospecifically centered, as dogs show less proficiency in using them with their conspecifics when compared to wolves. Reid (2009) describes another reason for dogs' ability to use human social cues: they have relied on humans for food, water, shelter, and consequently survival, throughout their evolution. This contrasts with species that share closer genetic ties with humans (e.g. chimpanzees) but do not rely on them for food. Such animals do not demonstrate the social capacities that dogs do when dealing with humans. Additionally, other domesticated animals that have shared shorter histories with humans show emerging signs of communicative abilities, suggesting that continued reliance on humans may promote these abilities (Reid, 2009).

In assessing the competing ideas of the various hypotheses, Reid (2009) turns to the separation between two phases of dog domestication: the initial evolution from their ancestral form and a secondary divergence to create numerous breeds. Because the differences seen among individual breeds do not follow a course of genetic separation from the wolf, it seems that sensitivity to cues would have been selected during the initial transformative phase of selection. Hare and Tomasello (2005) also assessed the viability of multiple social dog hypotheses and came to a similar conclusion. While some researchers tout enculturation with humans during ontogeny as the basis for dogs' capabilities, high early-life performance on object-choice tasks suggests that this is not the case. An alternative hypothesis that social skills are directly translated from the wolf without influence from domestication also do not account for the domestic dogs' abilities, because wolves do not perform well on these tasks. Hare and Tomasello

(2005) thus assert the likelihood that socio-communicative behaviors and lack of fear and aggression were selected during domestication.

Though individual caveats exist, the scope of evidence for socio-cognitive abilities in dogs largely supports this domestication hypothesis, and the hypothesis is perhaps best exemplified by Belyaev's fox experiment (Belyaev, 1979). This demonstration of evolution by selection presents a unique and vivid description of the effects of artificial selection on a wide range of genotypic and phenotypic traits. In an extensive multi-decade breeding program with silver foxes, selection for reproduction in the experimental group was based on tameness and lack of fear of humans. Over time, the generations of foxes that emerged were not only friendly, but they also demonstrated physiological and morphological changes such as piebaldness, longer molting cycles, and drooping ears. Additionally, reproduction transitioned from annual to biannual cycles, more closely resembling the domestic dog than their ancestral fox. Behaviorally, the foxes approached, licked, and played with their human caretakers much in the same way that dogs do. In large essence, the evolution of the silver fox by artificial selection provides a current-day progressive model of the multi-millennia domestication of the dog.

In order to further investigate the effects of domestication seen by Belyaev (1979), Hare et al. (2005) conducted a four-part experiment in which they compared object choice and preference behaviors across species. They found that when foxes and dogs were given human communicative signals to guide them to food reward in the object-choice task, experimental (tame) fox kits and dog puppies performed equally well and above chance. When experimental fox kits were compared to controls, they were much more likely than the control foxes to interact with an object that had been handled by a human and they also performed with higher accuracy on the object-choice task. Thus, the authors conclude that while selection for tameness is certainly not the only factor that may have led to the socio-cognitive abilities of the present-day dog, it is sufficient for such abilities and is exemplified in the silver fox.

Because dogs are an integral part of human society, it will behoove us to continue attempts to understand their perceptual and social world. Not only may this help us understand how to best ensure their welfare and enhance our interactions and training activities, but domestic dogs may also serve as socio-cognitive models for numerous contexts. Such contexts may include investigations of evolutionary change and human development. Additionally, from an evolutionary perspective, we have a unique and valuable opportunity to learn about the effects of selection on a variety of aspects of a single species – including, sometimes, those that may be wholly unexpected and unpredicted by current models. The potential questions, comparisons, and directions of research are numerous.

Socio-cognitive Research with Dogs

A great range of socio-cognitive tasks have been used with dogs in order to target general social competence, as well as more specific areas of this competence such as interspecific communication, attentional awareness, emotion recognition, and attachment. In nearly all cases, domestic dogs outperform other species and simultaneously track the behavioral repertoires of human intraspecific interaction. In reviewing the high performance by dogs on such socio-cognitive measures, one will notice a multi-dimensional arena for research in both dog and human social behavior. In the current research, we aimed to investigate variability in the attachment styles across dogs given their unique behavioral tendencies and training histories.

Behavioral Measures for Assessing Attachment. Much of the social attachment research with dogs approaches the dog-human relationship as analogous to the mother-child bond. The domestic dog presents itself as an apt model of human childhood development due to its shared environment, demonstrable social skills and reasoning, and position in familial structure. Additionally, much like preverbal infants, dogs are unable to communicate through verbal language with humans and must therefore use different modes to comprehend and transmit information. Further, dogs seem to place adult humans in the same fundamental and foundational social position as young children do. Virányi & Range (2014) describe the role of the parent in humans as one of safe haven and protection while simultaneously serving as a starting point for exploration, and they note that this seems to be true of dogs, as well. In fact, they cite a preference for familiar humans in dogs that is analogous to infants, as well as findings that puppies prefer human caretakers over their own mother dogs.

The well-known Strange Situation Test (Ainswoth, Blehar, Waters, & Wall, 1978), which provides a protocol for assessing attachment in children, has been used to assess the same behaviors in dogs. The Strange Situation Test measures attachment indices by placing the child or dog in a room with both his/her guardian and an unfamiliar person. Responses (e.g. crying) to the guardian leaving the room and the child/dog being left with the stranger are measured. In the case of human children, one of three attachment styles may be concluded from the Strange Situation Test. Topál, Miklósi, Csányi, and Dóka (1998) were the first to investigate attachment behaviors in dogs using the test. With 51 dog and owner pairs, the authors found that the test could be effectively translated from the human literature. The dogs were more active in play when their owner was present in the room with them and they were hesitant to interact with strangers, often ignoring them when their owners were not in the room. Instead, the dogs commonly stood at the door until their owners returned. Interestingly, dogs that were part of larger households exhibited fewer attachment and searching behaviors than dogs that were part of smaller households.

Kerepesi, Doka, & Miklosi (2015) investigated the importance of familiarity in human-dog interactions. They found that in situations that cause anxiety and fear, there is no substitute for the owner of a dog. Alternately, in situations that require obedience, dog are less partial to their owner. As would be expected, dogs do prefer their owners over unfamiliar humans, but the magnitude of such a preference is context-specific.

Emotion recognition is also a relevant and active part of dog-human communication. Merola, Prato-Previde, Lazzaroni, and Mashall-Pescini (2013) investigated perception of positive, neutral, and negative human emotion in dogs and found clear behavioral distinctions between contrasting valences. When a dog's owner expressed happiness toward one of two hidden boxes and fear toward the other, the dog was significantly more likely to approach the one tied to happiness. When neutral emotions were compared to negative emotions, dogs seem to be less discriminative, and this suggests that perhaps dogs are less familiar with displays of fear given by their owners than they are with displays of happiness. Interestingly, familiarity served as a key context-setter in this experiment, and dogs were much less likely to utilize emotional information given by strangers. Merola et al. (2013) leave this finding open to interpretation and further research, but suggest that this familiarity-based bias may be indicative of lack of motivation to use social information given by out-group members, an inability to distinguish between a stranger's emotions, or reduced attention allocation to strangers.

Emotional stimuli also create differences in tail-wagging behavior. Quaranta, Siniscalchi, and Vallortigara (2007) drew on the idea that although the tail is a medial organ, it should still be characterized by hemispheric control. In order to investigate the effects of emotive stimuli on tail-wagging lateralization, the researchers presented 30 dogs with four live stimuli through a screen: the dog's owner, an unfamiliar human, an unfamiliar dog with dominant temperament, and a cat. Dogs exhibited tail-wagging biases of varying degree to the right side when presented with their owner (greatest amplitude of wag), the unfamiliar human (medium amplitude), and the cat (lowest amplitude). On the contrary, a bias to the left side was seen for presentations of the dominant dog. Interestingly, these side biases fit well with hemispheric control of approach and withdrawal tendencies, with approachable stimuli tied to the right side of the body and left hemisphere of the brain and stimuli lending to avoidance tied to the left side and right hemisphere. The findings and procedure by Quaranta et al. (2007) present a significant opportunity for emotion research in the domestic dog, as well as other non-human animals.

Studies such as those outlined here have provided a strong foundation for the conclusion that attachment formation and maintenance are similar in humans and dogs, and that attachment is a strong quality of human-dog interspecific relationships. The current research aimed to assess the correlation between attachment to humans and a given dog's neural activation patterns and/or the dog's behavioral patterns as reported by their caretakers. In order to come to obtain a clear measure of attachment, we opted to conduct the unsolvable task with working dogs. This task is explained in the following section.

The Unsolvable Task

Related to assessments of preference for familiarity, the unsolvable task targets human attachment and dependency in the domestic dog. In this task, a dog is presented with a scenario in which it is unable to access a treat or toy that is beyond some sort of barrier. Generally, whimpering toward, pawing at, or looking toward a human in this situation is considered indicative of the dog drawing on its human partner for assistance in the task. Dogs that persist without seeking help are considered more independent and dogs that rapidly turn to humans are considered more attached.

Miklósi et al. (2003) found that when dogs and wolves were presented with an unsolvable version of a task that they had previously been trained on, dogs looked back to their handlers, but wolves did not. Marshall-Pescini, Valsecchi, Petak, Accorsi, and Previde (2008) found that highly trained dogs (e.g. agility) were more successful at opening a difficult box than untrained dogs, who looked back to their handlers more frequently. In a follow-up version of the task, Marshall-Pescini, Passalacqua, Barnard, Valsecchi, and Prato-Previde (2009) investigated training-based differences in attachment and found that agility dogs looked to their owners more than search and rescue and untrained pet dogs. This was likely due to the nature of agility training, which requires frequent feedback and visual communication from the handler. Horn, Virányi, Miklósi, Huber, and Range (2012) showed that dogs were more likely to look back at their owners in the unsolvable task when the owners had previously encouraged them to complete a solvable version of the task. Finally, Marshall-Pescini, Colombo, Passalacqua, Merola, and Prato-Previde (2013) directly compared dogs and young children in the unsolvable task and found that individuals of both species engaged in gaze alternation between the human and the inaccessible object. Thus, it again seems that dogs not only possess a strong social bond with humans but also that this bond is human-like in nature.

Given the efficacy of the unsolvable task in quantifying the preference for familiarity and the strength of the bond between human and dog, the unsolvable task was implemented herein. The task was conducted with military working dogs. This task is efficient for identifying dependency upon humans for problem-solving as well as default behaviors to familiar individuals. The unsolvable task measure provides multiple perspectives for assessing dependency and the strength of relationship a dog has with its human companion. That is, obtained data were analyzed in terms of frequency of showing behaviors, duration of help-seeking, and overall proportions of familiar versus unfamiliar preference. An additional benefit of this task is the prevention of within-session training effects, as the task utilizes comparatively fewer trials than other social measures and does not introduce any response-specific reward contingencies.

Dog fMRI

The use of fMRI provides an exciting and fairly unchartered area of comparative cognition research with domestic dogs. Explorations in dog MRI and fMRI began with the use of sedation to answer question about anatomy and physiology, primarily as a function of veterinary education and research. Such studies have provided knowledge of canine neural responsiveness, cognitive effects of aging, neuroimaging efficacy, and health viability. Bach et al. (2013) used fMRI to successfully identify neural regions associated with processing of auditory stimuli, as well as establish the efficacy of fMRI with dogs in regard to auditory stimulus presentation. Su et al. (2005) used longitudinal MRI to investigate the time course of neural correlates of canine cognitive decline (e.g. ventricular enlargement, lesions), strengthening the potentiality of the dog as a model of human aging. The efficacy of using high-field MRI to image the dog brain was explored by Martin-Vaquero et al. (2012), in which it was found the 3T MRI provided more consistent and reliable imaging data than did 7T MRI. In regard to health concerns surrounding MRI with dog subjects, Venn et al. (2014) published findings of post-scan hearing loss, emphasizing the need for hearing protection when imaging dogs in MRI environments.

Though prior cognitive research has been conducted with anesthetized dogs, the cognitive processes of their natural, attentive state are of great comparative interest. The use of anesthesia necessarily impedes attentiveness, as well as reduces rates of blood flow and respiration. The amalgamation of these reduced biomarkers of stimulus processing leaves much to be desired in the data set, as brain regions or activation patterns involved in cognitive processing may be minimized or lost altogether (Jia et al., 2014). In search of valid and viable findings, neuroimaging research with dogs has begun a transition to functional imaging using highly-trained dogs that do not require anesthesia for image acquisition. This movement in canine functional imaging has been pioneered by laboratories at Eotvos Lorand University, Emory University, and Auburn University. Here, I review the methods and findings published by each group (Table 1).

Bern, Brooks, and Spivak (2012) first published research on fMRI data acquisition in the awake and unrestrained domestic dog. The authors addressed three major challenges in using fMRI technology with dogs: subject motion, which distorts acquired data; use of anesthesia, which eliminates the viability of a cognitive assessment; and immobilization, which presents an ethical dilemma. To target these challenges, the authors developed a set of behavioral and technical methodologies for imaging dogs while they remained motionless, awake, and attentive to a cognitive task. Further, this methodological set was used to assess the reward-prediction error theory of dopamine release in dogs via use of reward signals and attention to activation changes in the ventral striatum. Specifics of this study are presented next.

Table 1

Previously Published Canine fMRI Studies

Paper	N	Task(s)	Stimuli	Area(s) of Activation
Berns, Brooks, & Spivak (2012)	2	Reward expectancy	(1) Reward hand signal (2) No-reward hand signal	Caudate (right)
Berns, Brooks, & Spivak (2013)	13	Reward expectancy	(1) Reward hand signal (2) No-reward hand signal	Caudate (left and right)*
Cook, Spivak, and Berns (2014)	12	Reward expectancy	(1) Reward hand signal (2) No-reward hand signal (a) Familiar Human (b) Unfamiliar Human (c) Computer	Caudate (left and right)
Andics, Gacsi, Farago, Kis, & Miklosi (2014)	11	Sound processing	(1) Human non-linguistic sounds (2) Dog sounds (3) Environment sounds	Both species: Primary Auditory Cortex, Medial Geniculate Body Humans: Superior Temporal Sulcus, Inferior Frontal Cortex Dogs: Perisylvian Regions
Jia et al. (2014)	6	Scent processing	(1) High concentration odor (2) Low concentration odor (3) No odor	Olfactory Bulb** Piriform Lobes** Frontal Cortex** Cerebellum**
Berns, Brooks, & Spivak (2015)	12	Scent processing	(1) Human (2) Dog (a) Familiar (b) Unfamiliar	Olfactory Bulb** Caudate***

Dilks, Cook, Weiller, Berns, Spivak, & Berns (2015)	8	Face processing	(1) Movie clips (2) Static Images (a) Human Faces (MC, SI) (b) Dog Faces (SI) (c) Objects (MC, SI) (d) Scenes (MC, SI) (e) Scrambled Objects (MC) (f) Scrambled Faces (SI)	Inferior Temporal Cortex (right)
Kyathanahally et al. (2015)	6	Resting state		Default Mode Network (anterior cingulate/medial preferontal areas dissociated from posterior cingulate)
Jia et al. (2015)	14	Scent processing	(1) odorants (a) zinc nanoparticles (b) gold nanoparticles	Olfactory Bulb***** Hippocampus*****
Cuaya et al. (2016)	7	Face processing	(1) Human faces (2) Everyday objects	Temporal Cortex Frontal Cortex Caudate Nucleus Thalamus

** greater activation in service dogs*
*** activation differences by concentration*
****activation for all scents*
***** greatest activation for familiar human*

Proof of concept. Two dogs were used as subjects in Berns et al. (2012), one of which had been previously trained in agility. Each dog was incrementally trained, using positive reinforcement, in a mock MRI scanner consisting of a replica of the head coil, scanner bore, and patient table. Additionally, the dogs were exposed to presentations of the scanner noises and sound levels that they would experience in the scanner. The cognitive task was trained by assigning reward conditions to each of two hand signals given by a handler: a hand held straight up signaled forthcoming presentation of food reward, and two hands held horizontally facing one another signaled no reward.

Once the dogs performed to criteria in the mock scanners, they were moved to true fMRI scanning in a Siemens 3T Trio over a period of 6 weeks. Initial scanning provided both an assimilation period and an assessment of image acquisition feasibility, followed by a subsequent session to optimize scanning parameters, and finally followed by image acquisition during the instrumental reward task. In this final session, the handler (positioned at the end of the bore) presented 10-second durations of the reward/no-reward task as previously trained.

Analysis of the obtained functional data focused on the head of the caudate in order to target the ventral striatum. The ventral striatum served as the predicted area for activation according to reward-prediction error learning, which anticipates dopamine release and corresponding neural activation of the ventral caudate upon expectation of reward. Reward and no-reward conditions served as the contrast of interest, revealing significant activation differences in the right caudate, though the meaning of the lateralized activation is unclear. These activation differences highlighted a distinct hemodynamic response for reward signal presentations as compared to no-reward signals, thus providing support for the notion that dopamine is released in response to unexpected events that signal future reward and, here specifically, a representation of positive reward prediction in the domestic dog.

Of additional note in regard to the research conducted by Berns et al. (2012) is the establishment of ethical guidelines for conducting fMRI with dogs. The authors highlight three conditions that they believe should be met in experimental design. First, no harm should be done to dog subjects, and this consideration includes the use of hearing protection (e.g. ear muffs) in order to attenuate the extreme sound pressure levels of MRI. Second, dogs should not be restrained and purpose-bred laboratory animals should not be used, as both of these scenarios violate the premise of the self-determination principle by which dogs should be allowed to exert free will over their actions. Finally, positive reinforcement training should be used when training dogs for scanning and behavioral tasks, and likewise punishment should be avoided.

Replication of the reward/no-reward task. Berns, Brooks, and Spivak (2013) followed their initial 2012 study of fMRI with dogs with an assessment of the replicability of their methodology. Further, the authors sought to evaluate the heterogeneity of caudate responses to the instrumental reward task with additional subjects and experimental improvements. In this replication, 13 dogs of various training background (e.g. service, agility, basic obedience) completed positive reinforcement training on the mock scanners (this time, with a mock knee coil instead of head coil) and the reward/no-reward task.

In this expanded subject set, 62% of dogs showed significant differential positive activation in the caudate for reward signals. These findings were consistent with Berns et al. (2012), however, substantial heterogeneity was found across subjects for overall caudate activation. Berns et al. (2013) discuss several potential reasons for this variability between subjects, including greater human attachment in service and therapy dogs, the inherent noise of imaging data, the difficult balance between imaging repetition and efficacy of the task, mislocation of regions of interest, and individual motivational differences. Interestingly, the authors note that when the dog fMRI data collected from the instrumental reward task is compared to that of humans', it may indeed be less variable than human caudate activity. Overall, this replication of awake, unrestrained dog fMRI methodology provided evidence for the efficacy of reliable training in demonstrating activation in the dog brain. Further, the results of this study and Berns et al. (2012) provide support for the possibility of dog models of human cognitive function.

Temperament and Stimulus Source. To further expand on their developments in dog fMRI, Cook, Spivak, and Berns (2014) modified their reward/no-reward task to assess activation differences driven both by subject temperament and stimulus source. The same dogs as used in Berns et al. (2013) were employed in this study, and all were evaluated for 14 factors of temperament (e.g. attachment, trainability, Hsu & Serpell, 2003) using the Canine Behavioral Assessment and Research Questionnaire (C-BARQ). Stimulus sources were divided equally among reward/no-reward hand signal presentations given by a familiar person or an unfamiliar person and digitized hand signal displays presented on a projection screen. Analyses revealed that across the subject set, the caudate was differentially active by condition, indicating further support for the implication of the ventral striatum in reward anticipation. Further, activations revealed that the dogs could generalize the meaning of the hand signals across stimulus sources. When C-BARQ temperament factors, particularly aggressivity, were taken into account, activation differences were found according to a stimulus source of familiar human versus unfamiliar human or projection by computer. That is, dogs with lower aggressivity levels showed greater activation for reward signals given by the familiar person than by the unfamiliar person or computer. Alternatively, dogs with higher aggressivity levels showed greater activation for reward signals given by an unfamiliar person or computer. Cook et al. (2014) note that, because the striatal response is dependent upon arousal and stimulus salience, higher aggressiveness correlates to higher salience for the novel situations of unfamiliar person and computer, and that lower aggressiveness correlates to lower anxiety and higher salience with a familiar person. In their conclusion, the authors stress the possibility of differences across dogs in their reactions to various contexts, and emphasize the need for consideration of this possibility when making claims from dog studies without temperament testing.

In all, these initial studies of functional imaging with dogs provided strong support for the opportunities presented by the merger of canine cognition with fMRI technology. The establishment of successful training and imaging techniques allows for the expansion of this research to involve more specific regions of interest along with a greater range of subjects and ontogenic histories. Notably, success with visually-based experiments provided an interesting opportunity to investigate processing in other sensory modalities.

Audition. Andics, Gásci, Faragó, Kis, and Miklósi (2014) have also used positive-reinforcement training to conduct fMRI studies with awake and unrestrained dogs. Here, the authors conducted comparative research into the function and location of voice-sensitive brain regions in dogs and humans. Because humans and dogs have long shared a natural environment, Andics et al. (2014) questioned how voice-sensitive regions in both populations would respond to conspecifics and heterospecifics, and whether they would show similar processing of emotional cues in these signals. Eleven dogs and 22 humans participated in scans during which an identical set of auditory stimuli was presented. This stimulus set consisted of human and dog vocalizations ranging in emotional valence along with environmental sounds and silence. The silence condition was used to functionally assign the auditory region of interest by contrasting silence activations against activations during sound presentation.

Cortical sound sensitivities were revealed in the perisylvian regions for the dogs and superior temporal sulcus and inferior frontal cortex for humans, and both species showed sensitivity in the medial geniculate body. In the dog brain, subregions were identified that activated maximally for dog vocalizations as well as to human vocalizations and environmental sounds. On the contrary, nearly all human auditory regions of activation were maximal for human vocalizations, although the medial geniculate body showed a maximal activation for dog vocalizations.

Olfaction. Jia et al. (2014) utilized positive-reinforcement training and fMRI with awake and unrestrained dogs to investigate olfactory processing and the effects of anesthesia on the quality of neural data. The authors note that there is a large body of literature pertaining to both the cellular and behavioral levels of olfaction in dogs, but little research has been done on the cognitive processes that underlie olfaction. Thus, their study aimed to serve as a comparison of the neural response in the brain to varying odor concentrations in awake versus anesthetized dogs. Six dogs served as the subjects for this study, and a specialized odorant delivery system was designed with MR restrictions and parameters in mind (e.g. elimination of ferromagnetic objects in the scanner room, motion control). This delivery system was used to precisely present 10-second periods of odorant to dogs across five randomized blocks.

Both awake and anesthetized dogs demonstrated strong activation in the olfactory bulb and bilateral piriform lobes upon presentation of both high and low concentrations of odor. However, the intensity of activations, as well as their spatial extent, was mediated by concentration, with larger activations for higher odor concentrations. Separating conscious dogs from anesthetized dogs were activations in areas including the medial, superior, and orbital frontal cortices and the cerebellum, all of which are tied to cognitive processes. Given the findings, the authors concluded that anesthesia degrades processing of odors and that the use of fMRI can and will provide a useful investigation into the neural substrates of the olfactory system.

Berns, Brooks, and Spivak (2015) sought to investigate the canine perceptual experience of socially-related stimuli via the processing odors of familiar and unfamiliar people and dogs. In order to investigate the driving social relationship between a human and dog, the authors again utilized the dopamine theory of reward-error prediction, hypothesizing that if the relationship between a dog and its most familiar person includes reward expectancy, then caudate activation will be greater when the scent of that person is being processed, as opposed to another person or a dog. The same dogs that were used in the prior research (Berns et al., 2012; Berns et al., 2013; Cook et al., 2014) were enlisted for this study. Additional training was needed to acclimate the dogs to smelling odors on a cotton swab while withholding approach. For presentation of swabs during scanning sessions, odors for the familiar and unfamiliar human were obtained from the armpits, and odors for the familiar dog, unfamiliar dog, and the dog's own self were obtained from the perineal-genital areas. In order to maintain compliance and motivation, the dogs were presented with interspersed reward trials during odor-presentation runs.

Analyses of the obtained imaging data focused on two regions of interest: the olfactory bulb and the caudate nucleus. The olfactory bulb was generally significantly activated by the task, and this activation was non-differential across all five odor types. However, the caudate nucleus showed differential activation according to odor type. For all dogs, the caudate was maximally activated for the odor of a familiar person, suggesting that a positive reward association is in place for the scent of a familiar human, even in their physical absence. Interestingly, service dogs once again stood out with greater overall caudate activation as compared to dogs with other histories.

Collectively, the studies conducted by Jia et al. (2014) and Berns et al. (2015) provide evidence for the efficacy of olfactory neuroimaging with dogs. The olfactory bulb has been consistently implicated in the processing of odors, and anesthesia and odor intensity are directly tied to neural activations. Additionally, reward-based processing of odorants was supported by activations in the caudate nucleus. Given the findings of these two studies, future research may look more closely at experience with and context of odor presentation and their effects on olfactory processing.

Face processing. In the first published fMRI investigation of face processing in awake dogs, Dilks et al. (2015) presented eight fMRI-experienced subjects (Cook et al., 2014) with movie clips and static images. The dogs viewed movie clips of human faces, scenes, objects, and scrambled objects, each for three seconds. In the static images condition, the dogs were presented with black and white images of human faces, dog faces, objects, scenes, and scrambled faces, each for 600 milliseconds. Imaging data was analyzed for six of the dogs and movie clip contrasts localized dog and human face processing to the inferior temporal cortex in the right hemisphere. The data also revealed significant category effect for static images when face images were compared to objects and scenes. Although there was not a category effect for scrambled face images, the response profile did not map onto the dog V1 area, suggesting that low-level feature processing does not account for the activation patterns seen in the temporal lobe. Rather, Dilks et al. (2015) conclude that the activations represent the first evidence of a face-processing region in dogs.

Cuaya et al. (2016) further explored face processing by dogs in fMRI using human faces and neutral objects. Seven dogs were presented with 50 images of human faces displaying neutral expressions and 50 images of everyday objects. The data obtained in this research yielded two activation clusters for the contrast of human faces versus objects. The first cluster was localized to the left temporal cortex and projected to the frontal cortex, caudate nucleus, and thalamus. The second originated in the right frontal cortex with projections to the right temporal cortex. The identified activation clusters were analogous to what has been shown across species, and the authors highlight the possibility that such findings in the domestic dogs represent a visual pathway that has been conserved over the course of evolution.

Resting State. Kyathanahally et al. (2015) used resting state fMRI to identify whether the default mode network (DMN), found reliably in humans and nonhuman primates but much less frequently in rodents, exists in the domestic dog. Resting state fMRI is conducted with subjects who do not perform any cognitive tasks, but rather lie still and relax. In humans, the DMN consists of precuneus, medial temporal, medial frontal, and inferior parietal cortical areas and is active during rest. This network has been implicated in cognition and self-referential processing and it has been found reliably in human resting state fMRI investigations. Additionally, this network's activity is depressed when a patient is under anesthesia (Greicius et al., 2008).

To assess the presence of a DMN in dogs and to understand the effects of anesthesia on its activation, Kyathanahally et al. (2015) scanned six dog subjects in both awake and anesthetized states. Seed-based and independent component analyses (ICA) were used and identified dissociation between the anterior and posterior regions of the DMN. Further, while this dissociation was seen for both awake and anesthetized dogs, the degree of dissociation varied. In all, this investigation into resting state fMRI with dogs revealed comparative differences in the traits of the DMN between humans/monkeys and dogs, namely localized anterior and posterior networks in dogs and a connected DMN in humans. The findings suggest differences in cognitive processing that are perhaps due to evolutionary time-course differences.

Moving forward. In summary, the research and findings discussed herein are representative of the current excitement and expansion of canine cognitive research into functional imaging. As interest and conceptual foundations in this area continue to grow, the cognitive processes and behavior of the domestic dog may be better linked to develop a comprehensive understanding of man's best friend. Further, such linking of cognition and behavior will allow for more informed comparisons to be made across species, as well as allow for greater understanding of the environment effects of domestication into the human social world. Though this area of research offers much promise, there are many challenges left to be addressed, both in respect to training and imaging methodologies and conceptual issues of cognitive investigation. In the following sections, we review those challenges most pertinent to future canine neuroimaging studies.

Although in-vivo scanning of domestic dogs is a fairly new but rapidly growing area of research, previous studies have established the viability and benefit of training dogs to remain awake and unrestrained during MRI. With the current research, we sought to continue to refine this methodology by utilizing highly trained personnel dedicated to scanning performance excellence. These trainers were able to produce positive compliance in dogs that was characterized by longer periods of stillness and comfort in the scanning environment without comprising the welfare of the dog or its functionality in other working contexts. Concurrently, the use of purpose working dogs ensured subjects that were highly motivated and willing to work with their handlers. This training was founded on positive reinforcement and comfort of the canine subjects and has been shown to be suitable in multiple studies conducted by Auburn University researchers.

Face Processing

Face processing is a vital component of human evolution and social cognition. By extracting information from faces, and individual is able to recall a person's identity, retrieve information about that identity, and use cues to aid in socialization (Paller et al., 2003). The body of face recognition literature has focused on both identity and expression of individuals. Across studies, the neuroanatomical structures and activation patterns of this type of cognition can be divided between a core system of processing and an extended system of processing, described by Haxby, Hoffman, and Gobbini (2000). The core system is activated by invariant traits that are used to identify an individual, and it is comprised of the inferior occipital gyri, the lateral fusiform gyrus, and the superior temporal sulcus. The extended system is activated by dynamic traits that facilitate communication, such as emotional expression or eye gaze, and its activation can be found in limbic regions (for emotion processing) as well as parietal regions (for processing of spatial information). Collectively, these systems provide the basis for keenly tuned human expertise in extracting information from faces.

Haxby et al. (2000) provide a concise model for the processing of face stimuli in humans as indicated by neural activations under conditions of core and expanded features. As noted, the core system includes three primary regions. The first region, the inferior occipital gyri, is implicated in early processing of facial features. Following this early perception are the processing of invariant traits in the lateral fusiform gyrus and initial processing of changeable traits in the superior temporal sulcus. The extended system is comprised of activations in four additional regions. Biographical identity information is processed in the anterior temporal areas, emotional content in the amygdala, insula, and limbic system, prelexical speech in the auditory cortex, and finally, spatial attention in the intraparietal sulcus.

Attachment Indices in Face Studies. To develop a framework for studying dogs' functioning in their relationship with humans, we look to similar research that has been conducted with humans. Stoeckel, Palley, Gollub, Niemi, and Evins (2014) used fMRI to compare responsiveness to child and pet dog images as seen by their mothers/owners. Participants completed behavioral measures for assessment of attachment to their children and dogs, after which they viewed images of their own child, their own dog, and unfamiliar dogs and children in the scanner and were asked to score them according to valence and arousal.

Attachment measures indicated that 93% of participants were extremely attached to their pet dog, considering him or her as a family member. Indeed, functional data revealed overlapping regions of brain activation including those associated with reward, emotion, and affiliation, namely the amygdala, hippocampus, and fusiform gyrus. However, two contrasts did reveal significant differences between familiar conditions. Images of one's child led to activation of the substantia niga/ventral tegmental area (implicated in reward and affiliation) whereas this pattern of activation was not seen with images of one's dog. And although the amygdala was activated by both conditions, images of one's dog led to greater activation of the fusiform gyrus than did one's child. Stoeckel et al. (2014) note that this may be due to the lack of language-based affiliation with dogs, as human-dog interaction may be more dependent on face perception to pick up on emotion, gaze direction, and identity.

Investigating Face and Emotion Recognition in Dogs. Through the current neuroimaging research, face processing in the domestic dog was investigated. We sought to locate the area(s) of activation involved when human face stimuli of varying familiarity and emotional valence are presented to dogs. Given the unique social life of the domestic dog, with close interaction both within species and with humans, we sought investigate how these bonds have influenced face recognition and processing. As a starting point, we knew that humans experience visual stimuli in different color and sharpness than domestic dogs (Miller & Murphy, 1995). However, when it comes to recognizing familiar faces, has our close bond with domestic dogs led to an ability to deal with familiar human faces in a similar way to that in which humans do? The answer to this question can provide further evidence for the mechanics of the human-dog bond and the effects of domestication, as well as provide a foundation for further canine cognition research to be conducted using fMRI. We hypothesized that dogs would demonstrate reliable activations in response to human faces in accord with both familiarity and emotional valence. We based these hypotheses on past neuroimaging research with both humans and non-human primates. Following from human research, regions of interest included analogous regions to those associated with face processing in humans (e.g. the core and extended systems). Further, non-human primate work suggested that we might find differential activation in the hippocampus that was mediated by familiarity (Sliwa, Planté, & Wirth, 2014), as well as differential activation in the amygdala that was mediated by emotional valence (Hadj-Bouziane et al., 2012).

Applicability to Working Dogs

Humans and dogs have worked alongside one another for thousands of years. Though the jobs were not specialized in the manner that they are today, dogs have long served to guard and protect livestock as well as assist in hunts (Miklósi, 2007). Today, domestic working dogs play vital roles in a variety of search, detection, patrol, and service work. In filling these roles, they often perform above and beyond human abilities. Cobb, Branson, Mcgreevy, Lill, and Bennett (2015) define a working dog as one which is “operational in a private industry, government, assistant, or sporting context,” while noting that these dogs may also simultaneously serve as human companions. Currently, there is a need for assessment and identification of young dogs that are well-equipped to fill these roles. Although the methodological challenges of this undertaking are great, an organized and effortful plan of action will ensure optimal use of resources and welfare of dogs filling working roles.

Demands of Working Dogs. The skills and abilities needed for an individual working dog vary greatly by job. Police dogs may participate in patrol duties such as apprehension and crowd control, tracking criminals, or recovering stolen items (Slabbert & Odendaal, 1999). Within military work, the broad scope of duties a dog may fill includes combat support, personnel and building sweeps, and route clearance. Detection work within the United States Armed Forces is highly specialized and may be preceded by certifications in mine detection, combat tracking, narcotics detection, explosives detection, and specialized search training (U.S. Army, 2013). In other arenas, specialized search dogs may be used to detect a variety of targets, from termites to melanomas. In any case, search dogs are required to cover large areas for long period of time without decline in energy or performance, often unaccompanied by human handlers (Rooney, Gaines, Bradshaw, & Penman, 2007).

Outside of police and military work, domestic dogs often fill substantial roles as companions through service training. Dogs may be used to provide emotional support following psychological trauma, and may be used as catalysts for discussion in psychotherapy (Svartberg, 2002). Dogs across the world are trained as guide dogs for the blind and assist with daily tasks that would otherwise require a loss of independence for their human companion. More recently, dogs have been trained in service for veterans struggling with Post-Traumatic Stress Disorder (PTSD), turning on lights, conducting sweeps for intruders, and provided physical support in crowds (Yount, Olmert, & Lee, 2012). In one study, a dog facilitated vocal speech in a patient with aphasia (LaFrance, Garcia, & LeBreche, 2007).

The working dog industry has been and is continuing to grow at a rapid rate, with dogs being trained in increasingly complex duties and the breeding programs producing greater numbers of puppies. Producing dogs for work in any number of jobs requires genetic selection, care and raising of puppies, selection and testing based on ability for the job, medical care, housing, handler selection and training, and work end-point management. Consequently, there is a high cost associated with the production of working dogs. For example, a single guide dog for a blind individual may cost between $10,000 and $20,000 to produce (Batt, Batt, Baguley, & McGreevy, 2008). Wherever there are shortfalls within components of the larger production system (e.g. puppy selection or early training experience), inefficiencies occur and can often lead to substantial reduction in success rates (Cobb et al., 2015).

In turn, low success rates lead to large deficits and lost revenue for working dog organizations. Maejima et al. (2007) noted that while Japan employs upwards of 100 dogs for prevention of drug smuggling at any time, 60 are trained each year with only a 30% success rate. Within Japan's service dog industry, rates of only 30% to 40% success in guide dog training and certification have been reported (Dalibard, 2009). Similarly, the Swedish Armed Forces have reported production rates of 200 puppies per year with an average of approximately 50 successful dogs per 200 (Wilsson & Sinn, 2012). The South African Police Service Dog Breeding Centre has reported failure rates of 70% (Slabbert & Odendaal, 1999). Across the board, organizations within the working dog industries consistently report failure rates of 50% to 70%. Because working dogs cost several thousands of dollars to produce, the losses seen by these organizations could be considered excessive.

Assessing and Identifying Traits of Optimal Working Dogs. The identification of individual ideal behavior traits in working dogs is muddied by a lack of clear and concise definition of these traits. For example, “drive” is a commonly valued and measured trait across working dog roles, but its definition varies by context, meaning something different for work in contexts of scent detection, livestock herding, and racing (Cobb et al., 2015). A first step for researchers to pinpoint identifiers of working dog success will require a significant movement to thoroughly define global behaviors rather than using niche-specific terms and tendencies in behavior to qualify traits. This may be done with comprehensive consultation with working dog organizations across fields and with repeated measurement of young dogs based on the agreed upon criteria for traits of interest. Ultimately, each behavioral trait should be defined globally from a perspective of real-time demonstration by dogs, regardless of training, as well as operationally from a perspective of exhibition of the trait in individual working contexts. Further, these traits should be designed to be identifiable and measurable across fields and assessor experience levels.

Assessments of ability are important not only for the efficiency and success of working dog programs, but also for the viability of individual animals. Mizukoshi, Kondo, and Nakamura (2008) note that training dogs that do not possess the necessary abilities for a given job presents a welfare concern. Aside from the strain that the training may place on an animal that is not equipped for work in a given context, the time spent on that training may prevent them from socialization or learning of behaviors that would help them succeed in a different working context, or perhaps as a family pet. Given this risk, assessment techniques need to provide early detection of behavioral traits that prevent suitability for a job in order to release the dog from the system at as young an age as possible.

It might seem most efficient to investigate a genetic means of assessment and selection of working dogs. Dopamine receptor D4 (DRD4) has been implicated in novelty-seeking and serotonin in anxiety levels in dogs, and therefore could feasibly influence working life success by pushing a dog toward certain extremes of behavior. Maejima et al. (2007) sought to investigate differences in working dog success based on the analysis of these genes, but no significant differences in their composition or expression were found that tracked onto the genetic makeup of the dogs. Further, breeding as a basis for working dog stock is complicated by the fact that differences in working dog ability are generally due to behavioral differences rather than sensory or morphological characteristics (Sinn, Gosling, & Hilliard, 2010), and even within selection of breed traits, idiosyncratic differences can always be observed (Wilsson & Sinn, 2012).

Puppy Tests. Researchers have long sought to pinpoint the earliest age at which a dog's trait repertoire is stable enough to serve as an identifier for working dog success, reaching as far back as 1963 in the selection of guide dogs for the blind (Pfaffenberger, 1963). Commonly known as puppy tests, assessments of these early identifiers have been called one of the "holy grails" of dog research (Miklósi, 2007). Unfortunately, studies of puppy tests have yielded inconsistent results to date, rarely demonstrating consistency in assessment components, dog assessment age, or attempts at methodological validation (Asher et al., 2013). Further, many puppy tests are wholly irrelevant to a dog's general abilities or the tasks at which working dogs need to succeed. For example, these tests may involve suspending a puppy in the air, restraining it on its back, or pinching it to look for so-called signs of dominance (Slabbert & Odendaal, 1999). However, such scenarios are not only foreign to the behavioral repertoire of their species, but they are also not experienced by dogs in their working duties. In order to be useful, assessments of puppyhood behaviors should be identical to, or at least correlate with, specific behaviors that are regularly utilized by working dogs in a given context. For example, if a support dog should maintain frequent eye contact with its handler, then the tendency for turning gaze to a human face should be measured during puppyhood.

Svobodová, Vápeník, Pinc, and Bartoš (2008) attempted to create a measure of puppyhood behavior that would predict later certification in police work. In an assessment of 206 German Shepherd puppies tested at the age of seven weeks, they found that weight, predation attitude, noise responsiveness, and movement predicted the success of the dogs in passing certification. Similarly, Slabbert and Odendaal (1999) achieved predictability of future police work success in early-life testing with 167 puppies. Here, they began socialization activities at the age of four weeks and began assessments at the age of eight weeks. Retrieval tests at eight weeks and aggression tests at six and nine months merged to predict 81.7% of unsuccessful working dogs and 91.7% of successful working dogs. Thus, the authors were able to demonstrate prediction of adult behavior in police dogs. Drawing from this literature, it seems that general behaviors of confidence and energy are ideal for these dogs, but again a problem of clear definition and validation have been raised by multiple studies utilizing different methods to target already convoluted terms.

Asher et al. (2013) used a Puppy Profiling Assessment to target the responsiveness and confidence needed for guide dogs by testing puppies for responses to several untrained scenarios (e.g. following a human or chasing a prey-like figure). In their study, they found that dogs that scored low on confidence and responsiveness were less likely to qualify for the job. Highlighting the utility of a measures-based cutoff point, they found that puppies scoring less than -1.5 on their assessment corresponded to 93% of dogs that were unsuccessful due to behavior problems. Working toward a similar goal, Maejima et al. (2007) conducted a long-term subjective evaluation of nearly 200 Labrador Retrievers, measuring general activity, aggression, anxiety, obedience, interest and concentration of potential search dogs. They also conducted single-test measures in which the dogs were tested in mock scent detection scenarios in relevant areas such as baggage and mail. Overall, they found that scores on a drive for work measure were most indicative of success and suggested using such a measure for developing cutoff points for dog selection, as 53.3% of the unsuccessful dogs and 93.3% of the successful dogs were identified by the drive score. As such, while we may not expect to see 100% success rates with dogs selected for according to behavioral test criteria, there is good reason to think that high levels may be achieved simply by pinpointing ideal score cutoffs.

Rooney et al. (2007) assessed the behavior of 26 Labrador Retrievers slotted for scent detection work over the course of 10 weeks, with measures for overall ability, individual behavioral traits, a prediction of future success by the handler, and the Standard Search Assessment for locating hidden substances in a room. Here, they found that general ability scores and the number of false target indications given by the dogs in the search assessment were predictive of later working success. Further, they found that overall, aggregate scores rather than single-item traits were more predictive of success. This would again suggest that the ideal battery of behavioral assessment in working dogs might include a minimum score for selection and inclusion in training.

Svartberg (2008) tested more than 2,600 Swedish Armed Forces dogs between the ages of 12 and 18 months. They found that successful dogs were more active, confident, and playful than their unsuccessful counterparts. They postulated that dogs low in fear may be less susceptible to distraction and dogs high in playfulness may be more likely to be motivated by the opportunity for active reward. However, additional training may supplement these tendencies in higher fear and lower play dogs. Regardless of the remedial measures that could be taken, negatively-directed behaviors such as fear or startle might need to be accounted for in separate assessments, where higher scores correspond to greater extremes of these behaviors. Such scores could serve an alerting purpose for handlers to either instate a correction plan or, in the case of highest-stress working roles, suggest movement of the dog to a different working context.

One problem with the identification of the appropriate age for puppy testing is the variability in development across breeds. Because the motor and perceptual abilities of puppies are different across breeds, and respectively different between individual dogs, these abilities may not be fully developed if a test parameter requires assessment at a single day- or week-age interval. To combat this, Miklósi (2007) proposed three criteria for designing an assessment of dogs at a very young age. First, behavioral indicators of motor and perceptual ability need to be established and defined such that within a given dog, the first emergence of the ability, the rate of its development, and its stability can be recorded. Second, the design of a given assessment of ability should target the characteristics of interest with multiple behavioral tests that reveal that ability. Further, these tests should be conducted a second time on the following day. Third, given the considerations to development and tests revealing the ideal characteristics, multiple repetitions of the tests should be given across the course of development in order to create a test battery indicative of the desired trait in a single dog. Overall, it seems that puppy testing will require significantly more time that what has been allotted in the past. That is, single-shot assessments will likely not suffice, and researchers should gear their efforts toward development of long-term repeated measures designs for identification of behavioral traits in individual dogs.

In general, puppies go through a sensitive period for socialization and learning from two-and-a-half weeks to 12 weeks. Relationships with other dogs and other species such as humans are formed during this developmental period, and depriving a puppy of exposure to novel stimuli during this time may increase fearfulness later in development (Miklósi, 2007). Thus, a benefit of frequent early-life testing is the concurrent enrichment the tests provide for the dog. These tests expose the dog to several physical and social experiences that they might otherwise miss in a kennel environment, many of which will be part of their everyday lives and jobs as working dogs. Further, frequent test interactions between handlers and dogs allow the handlers to understand the developmental course of individual dogs. In the case that the puppy is scoring at sub-par levels, then extra training or care may be provided to improve the behavioral repertoire of that animal (Miklósi, 2007). Unfortunately, because many puppy tests are currently given between the ages of nine and 18 months, many dogs have already entered high-cost training programs after their behavioral traits have been solidified (Asher et al., 2013). Again, time is a vital factor in improving the success of working dogs, and hesitation and postponement of behavioral measures will only work against training efforts.

Eliminating Problems in Welfare, Selection, and Training. Aside from the difficulties of identifying dogs that possess the ideal behavior traits for a working life, there are many challenges posed by the training and daily lifestyle of a working dog. Sinn et al. (2012) note that many (possibly all), behavioral characteristics can be molded by the environment, and in many cases the juvenile environment and lifestyle of working dogs is both sterile and stressful. Thus, working dog organizations stand to benefit from designing training methodology and housing areas that contribute to the well-being of working dogs. Changes to be made should be founded on the goal of providing cognitive, social, and environmental enrichment. Additionally, training and selection measures should be validated, as the current body of evidence is not easily translatable to new contexts and new populations of dogs.

Aside from the ethics involved, Rooney et al. (2007) demonstrated a link between welfare and working performance in search dogs, with decrements in performance and learning ability for dogs that were stressed. Pfaffenberger (1963) stated that boredom comes from a lack of things to do and thus prevents dogs from achieving their potential. Rooney et al. (2009) suggest that welfare may be improved by increasing socialization with humans, designing kennel environments that are more interesting for dogs, and introducing potentially stressful situations in a positive manner. Thus, while some components of the arrangement of kennel living may be necessarily stressful, positive associations (perhaps aligned with food or praise) may alleviate the negative responses experienced by dogs. As an added avenue for alleviation of unpleasant experience, Cobb et al. (2015) note that species-specific social contact reduced stress. Such a stress solution could be easily implemented in kennel play yards, as these environments are certainly not lacking in potential for species-specific interaction. Additionally, well-known stress-alleviating activities for dogs are play and exercise, and these would follow suit with implantation of dog play time (Slabbert & Odendaal, 1999).

Selection Measures. A great variety of traits and characteristics may be sought by different organizations under different conditions. For example, dogs at Lackland Air Force Base are tested for motivation to engage in goal-directed behaviors in exchange for positive reinforcement (Sinn et al., 2010). However, problems with behavioral measures often arise with regard to their generalizability (Duffy & Serpell, 2012), as many tasks included in a test may not actually occur outside the testing arena or may not share timing, stress levels, or distractions. Assessments should be designed around comprehensive dog ability, accounting for all aspects of the job (Rooney et al., 2007) and should accordingly replicate the psychological and physical demands of that job.

There is a need for reliability and validity within assessments of working dog ability, and these should be established through both inter-rater and test-retest reliability, as well as predictive validity (Sinn et al., 2010). Rooney et al. (2007) targeted and eliminated problems of interobserver agreement by establishing clear definitions for rated behaviors and testing them for clarity beforehand. Additionally, the observers were well versed in assigning scores to many different dogs, contributing to internal reliability of rating.

Duffy and Serpell (2012) addressed the validity issues of single-test observations by relying on individuals who had extensive contact with the dogs as puppy raisers. In this assessment, the C-BARQ was used for more than 7,000 dogs at the ages of six and 12 months. The researchers found significant differences between dogs that became successful as guide dogs and those that were released from their programs on 26 of 27 measures at each age. Though the results were promising as to identifying traits that lead to success, the authors caution that this shouldn't be the sole criterion for guide dog selection and that validity and reliability must still be established.

The method of scoring behaviors is also one of debate, and perhaps the best solution is to use both objective and subjective rating systems in conjunction with definition-based coding. Wilsson and Sinn (2012) explain that assessments using behavioral coding function by narrowly defining observable behaviors and scoring those behaviors in a direct, cumulative manner. On the other hand, ratings methods require observers to intuitively assess a dog's position on a given scale. These ratings can be further broken down into subjective, history-based measures and objective, test-based measures. Using single-test samples eliminates handler bias from experience, but this method must also ensure that the measures target typical, everyday performance. In comparing the predictive validity of the two ratings systems, Wilson and Sinn (2007) found that there was little difference between the two.

Given the high costs and waste of resources incurred by failures in selection and training of working dogs, there is an urgent need within the industry for reliable and effective measures to assess long-term temperament, physical and cognitive ability, and trainability. As such, the interdisciplinary arena of canine performance science has arisen with the goal of ensuring research-based quality and welfare of working dogs (Cobb et al., 2015). In this regard, the current research aimed to identify neural and behavioral correlates of successful working dogs. We link behavioral methodology with functional neuroimaging to identify behavioral and neurological markers of successful working dogs.

While training programs and working purposes vary among working dog organizations, fMRI methodologies can be developed to identify common activation areas and patterns among dogs that pass rigorous training and succeed in the workforce. Discovering such biomarkers may lead to better standards of identification, training, and treatment of dogs intended for working roles. An endophenotype for a specific working dog role may be developed in a stepwise fashion by using behavioral assessments to identify the most viable behavioral tendencies to fulfill the role and then correlating scores on such identifiers with brain activation data, such as neural responsiveness to target odors, auditory cues, or visual markers. On a long-term scale, such a combination of neuroimaging and behavioral profiling could drastically enhance the efficiency and ethical soundness of the working dog industry.

Chapter 2: Experiments

Experimental Overview

The three experiments described herein aimed to, (1a) develop a reliable and valid stimulus set of emotion expression in human faces specific to the experience of the subjects, (1b) identify activation patterns specific to familiarity and emotion recognition in the domestic dog, and (2) identify neural and behavioral correlates of the dog-human bond, as well as correlates of successful working dogs. Organization of the three experiments is seen in Figure 1. The first phase of Experiment 1(a) served to validate the visual stimulus sets to be used in the fMRI experiment. The second phase of Experiment 1(b) was conducted with working dogs as a neuroimaging investigation into the human-dog bond. Experiment 2 incorporated an established behavior measure, the unsolvable task, to provide an assessment of working dog bias toward familiar trainers as well as produce a data set for correlation between neural activation from Experiment 1(b) and behavior.

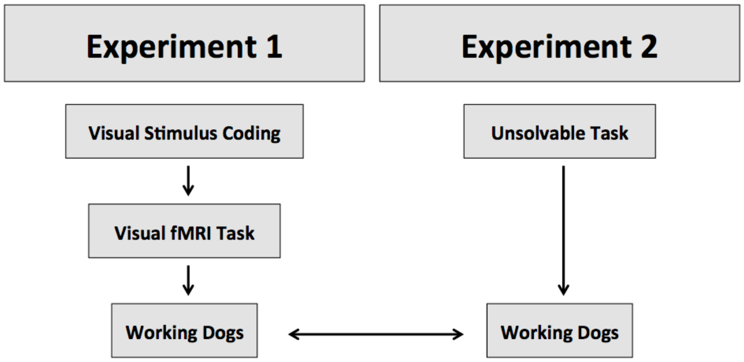


Figure 1. The flow of the experiments. The visual fMRI task was preceded by normalization of the stimulus set. Dogs participated in both the visual fMRI task and the unsolvable task.

Experiment 1a: Visual Stimulus Development

A variety of stimulus sets have been normed for affective quality in human populations. These sets may apply broadly to target global affective quality (e.g. Lang, Bradley, & Cuthbert, 1997) or may be tailored to specific populations such as the military (Goodman, Katz, & Dretsch, 2016). Normed stimulus sets are standardized using ratings from a large number of scorers on one or more dimensions. For example, the International Affective Picture System (IAPS) utilized three dimensions of assessment: valence, arousal, and dominance. Scores were obtained using a continuous scale on which raters could select the most accurate position on the scale, ranging from a sleepy figure to an excited figure for the arousal dimension, frowning to smiling for the valence dimension, and large self to small self for the dominance dimension.

Because dogs cannot overtly report perception of emotional valence, the need for a normalized stimulus set was amplified for the current research. By using scored stimuli, we were better able to ensure that the dogs were presented with the emotions we intended and to which we correlated the obtained data. The stimulus set used in the visual fMRI task was comprised of human faces that varied along dimensions of familiarity and emotional valence. It was critical to develop a set of stimuli specific to the dogs in this study in order to assess familiarity. Faces of trainers were used to create the familiar condition and faces of volunteers comprised the unfamiliar condition. Regardless of familiarity, each human displayed positive (happy), neutral, and negative (angry) emotions for still images and videos.

Method

Participants

Participants for visual stimulus development included the dogs' familiar trainers and unfamiliar humans recruited via Auburn University and the Auburn, AL community.

Stimulus Acquisition

Still Images. The still images condition consisted of familiar faces (handlers/trainers) and faces of unfamiliar individuals. Within these conditions, the models demonstrated positive/happy expressions, neutral expressions, and negative/angry expressions. Models were encouraged to display as much emotion as possible for each photo. Images were captured using a Canon Rebel XT 8-megapixel DSLR camera and were edited and processed in Aperture. Images were cropped to 600 x 600 pixels framed around the face and neck and were saved as JPEG files.

Videos. For the video condition, we again utilized familiar and unfamiliar individuals displaying positive, neutral, and negative emotions. In the positive condition, models said, “Good dog!” repeatedly in an excited tone and with a great deal of happy expression. In the neutral condition, we avoided the use of potential ‘trigger words’ and asked the models to repeat, “We’re gonna do this. We’re gonna do that.” They did this in a monotone voice with no emotion expression. In the negative condition, models said, “Bad dog!” repeatedly in a forceful tone and with anger expression. Videos were captured using a GoPro Hero 3 camera and were edited and processed in Quicktime for Mac. Videos were adjusted 1024 x 768 pixels framed around the face and neck and were saved as AVI files.

Scoring

In order to be sure that the dogs were presented with images and videos of the intended emotional valence, raters scored each stimulus. They were asked to identify the emotion displayed as well as the degree of that emotion on a scale from “Very Low” to “Very High” (Figure 2). Mean scores were calculated for individual images, resulting in a composite score for each stimulus ranging from -5 (angriest) to +5 (happiest). To develop the initial still images set, we matched each of the familiar handlers' images to the unfamiliar image with the closest valence score. To develop the initial video set, we retained each of the familiar handlers' videos and paired then with the corresponding unfamiliar video that was rated to have the most extreme valence score (i.e. closest to -5 for negative, 0 for neutral, and +5 for positive).

1.
Please select the emotion shown here and rank it on a scale from 1 to 5.

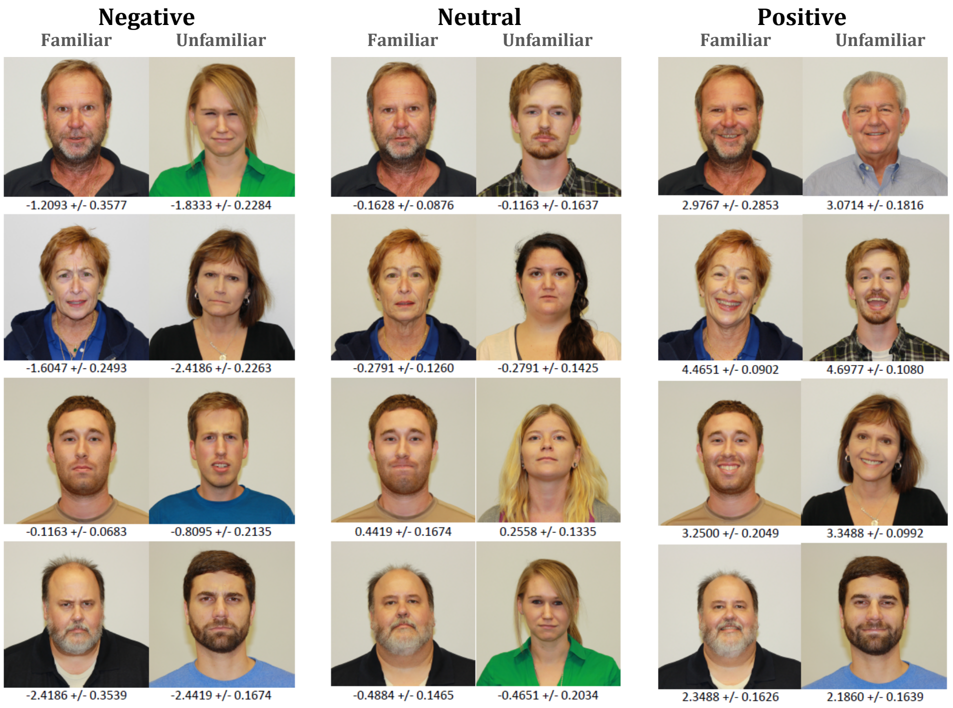
Please select the emotion shown in each photo/video. Then, rank the degree of that emotion.

1	☐Angry ☐Neutral ☐Happy (1) Very Low (2) Low (3) Medium (4) High (5) Very High

Figure 2. A sample image and scoring form of the emotional valence scoring procedure. The corresponding scoring form can be seen in Appendix A.

Stimulus Sets

The stimuli included in the initial still images set varied according to dog group (due to varying familiarity with trainers), but each stimulus set included 24 images (Figure 3). Within each set, there were four positive familiar images, four neutral familiar images, four negative familiar images, four positive unfamiliar images, four neutral unfamiliar images, and four negative unfamiliar images. Likewise, the stimuli included in the video set varied according to dog group (trainer familiarity variance), but each set included 24 videos (Figure 4). Within each set, there were four positive familiar videos, four neutral familiar videos, four negative familiar videos, four positive unfamiliar videos, four neutral unfamiliar videos, and four negative unfamiliar videos.



Mean valence values on a scale from -5 (most negative) to +5 (most positive) from 43 raters.

Figure 3. A sample still images stimulus set. Images of unfamiliar humans were matched to images of familiar humans according to emotional valence score. The final stimulus set consisted of eight positive images, eight neutral images, and eight negative images.

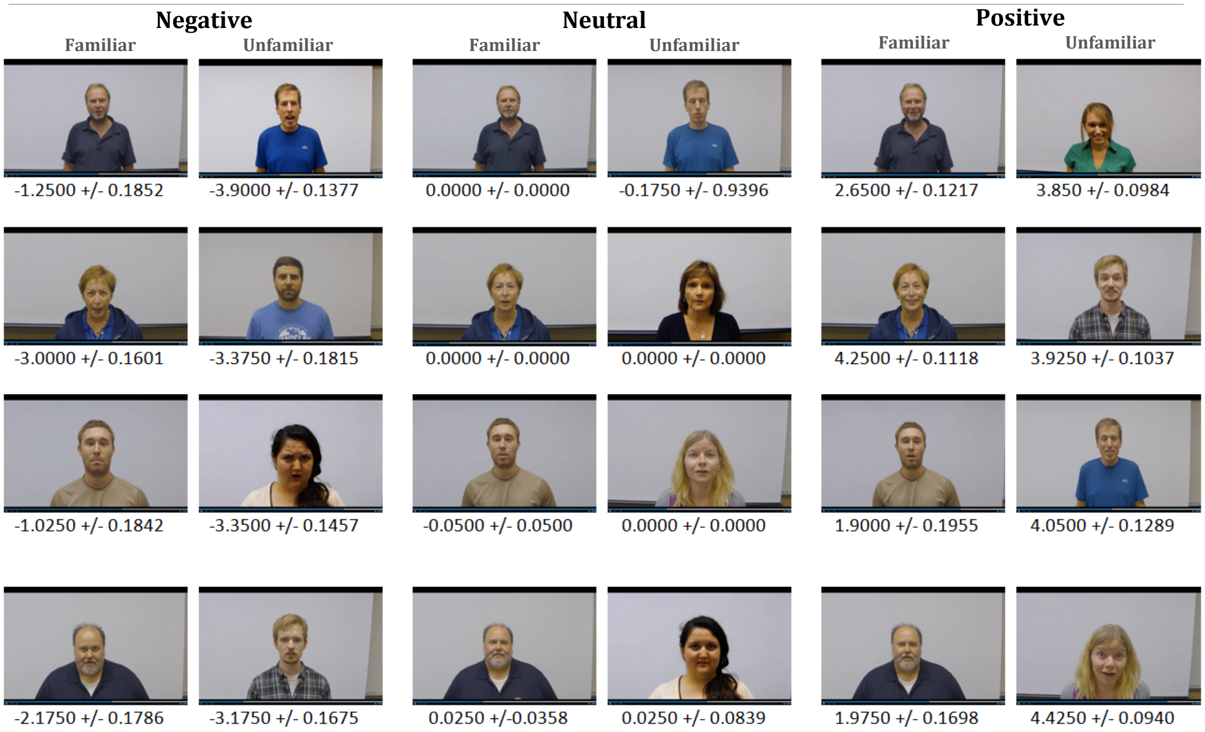
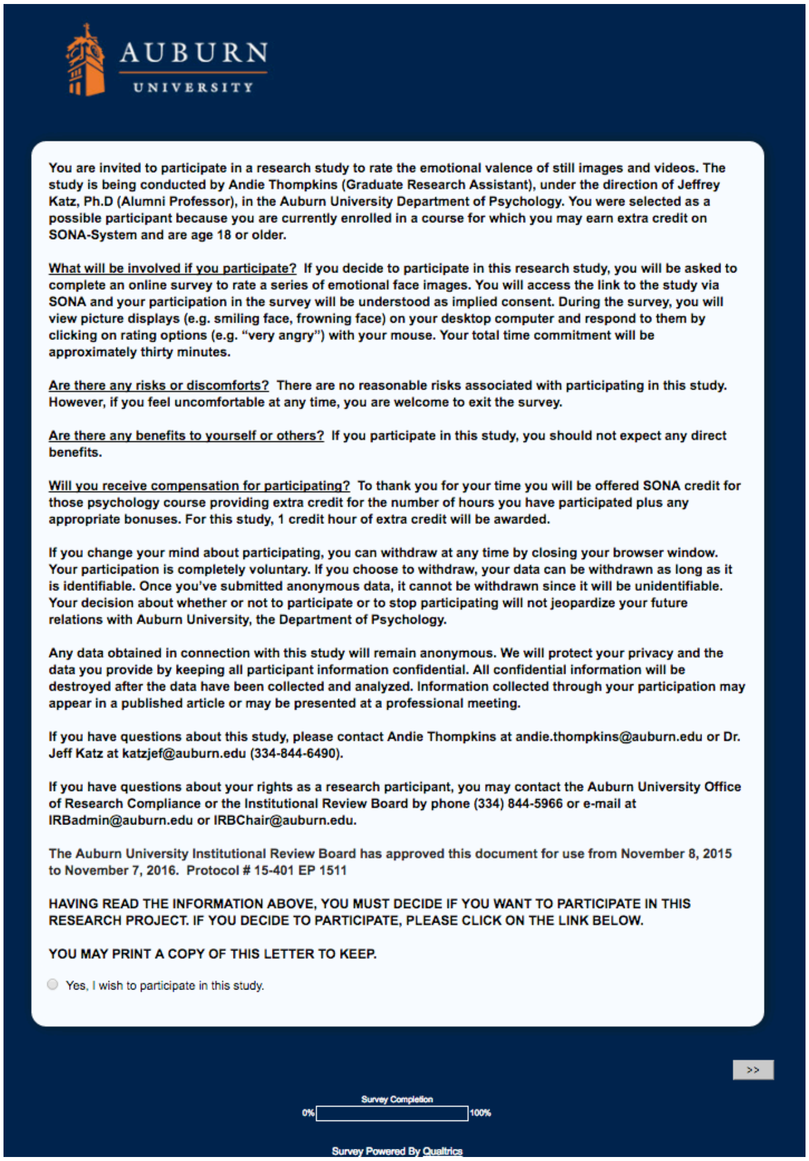


Figure 4. A sample video stimulus set. Videos of familiar humans were paired with videos of unfamiliar humans that hold the most extreme valence scores. The final stimulus set consisted of eight positive videos, eight neutral videos, and eight negative videos.

Expanded Stimulus Sets. An expanded stimulus library was developed using the methods described above. New iK9 personnel were included in this stimulus library. Ratings by 88 Auburn University undergraduate students were acquired online using a Qualtrics survey for which they earned extra credit via the Department of Psychology Research Participation Program. Participants were asked to score each image according to emotional valence using the same method applied to the original stimulus set (Figure 5, 6). Responses were averaged (N = 88) to develop a final valence score for each stimulus. These scores can be seen in Table 2.



You are invited to participate in a research study to rate the emotional valence of still images and videos. The study is being conducted by Andie Thompkins (Graduate Research Assistant), under the direction of Jeffrey Katz, Ph.D (Alumni Professor), in the Auburn University Department of Psychology. You were selected as a possible participant because you are currently enrolled in a course for which you may earn extra credit on SONA-System and are age 18 or older.

What will be involved if you participate? If you decide to participate in this research study, you will be asked to complete an online survey to rate a series of emotional face images. You will access the link to the study via SONA and your participation in the survey will be understood as implied consent. During the survey, you will view picture displays (e.g. smiling face, frowning face) on your desktop computer and respond to them by clicking on rating options (e.g. "very angry") with your mouse. Your total time commitment will be approximately thirty minutes.

Are there any risks or discomforts? There are no reasonable risks associated with participating in this study. However, if you feel uncomfortable at any time, you are welcome to exit the survey.

Are there any benefits to yourself or others? If you participate in this study, you should not expect any direct benefits.

Will you receive compensation for participating? To thank you for your time you will be offered SONA credit for those psychology course providing extra credit for the number of hours you have participated plus any appropriate bonuses. For this study, 1 credit hour of extra credit will be awarded.

If you change your mind about participating, you can withdraw at any time by closing your browser window. Your participation is completely voluntary. If you choose to withdraw, your data can be withdrawn as long as it is identifiable. Once you've submitted anonymous data, it cannot be withdrawn since it will be unidentifiable. Your decision about whether or not to participate or to stop participating will not jeopardize your future relations with Auburn University, the Department of Psychology.

Any data obtained in connection with this study will remain anonymous. We will protect your privacy and the data you provide by keeping all participant information confidential. All confidential information will be destroyed after the data have been collected and analyzed. Information collected through your participation may appear in a published article or may be presented at a professional meeting.

If you have questions about this study, please contact Andie Thompkins at andie.thompkins@auburn.edu or Dr. Jeff Katz at katzjef@auburn.edu (334-844-6490).

If you have questions about your rights as a research participant, you may contact the Auburn University Office of Research Compliance or the Institutional Review Board by phone (334) 844-5966 or e-mail at IRBadmin@auburn.edu or IRBChair@auburn.edu.

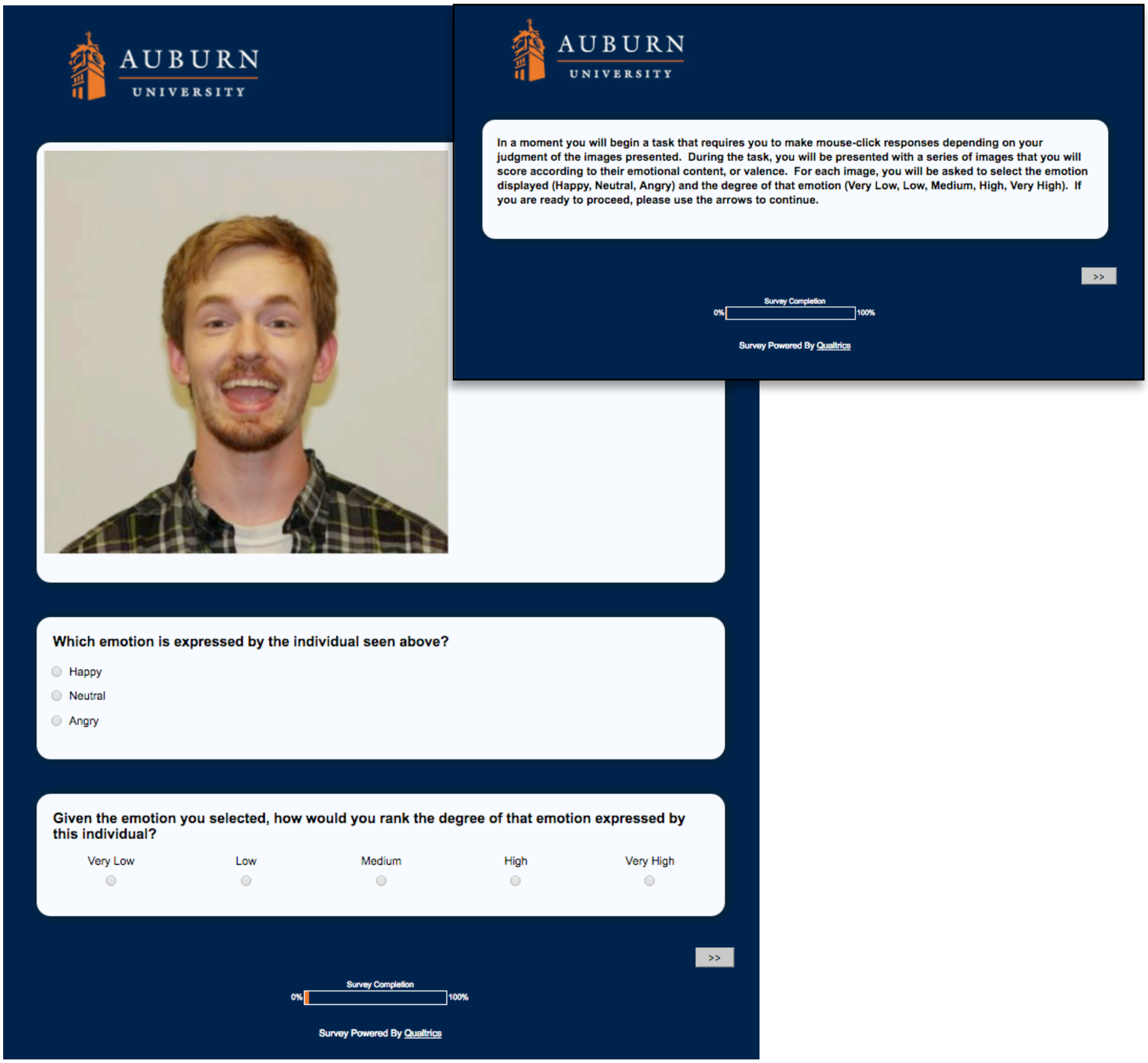
The Auburn University Institutional Review Board has approved this document for use from November 8, 2015 to November 7, 2016. Protocol # 15-401 EP 1511

HAVING READ THE INFORMATION ABOVE, YOU MUST DECIDE IF YOU WANT TO PARTICIPATE IN THIS RESEARCH PROJECT. IF YOU DECIDE TO PARTICIPATE, PLEASE CLICK ON THE LINK BELOW.

YOU MAY PRINT A COPY OF THIS LETTER TO KEEP.

○ Yes, I wish to participate in this study.

Figure 5. Consent form as seen by online stimulus rating participants.



In a moment you will begin a task that requires you to make mouse-click responses depending on your judgment of the images presented. During the task, you will be presented with a series of images that you will score according to their emotional content, or valence. For each image, you will be asked to select the emotion displayed (Happy, Neutral, Angry) and the degree of that emotion (Very Low, Low, Medium, High, Very High). If you are ready to proceed, please use the arrows to continue.

Which emotion is expressed by the individual seen above?

○ Happy
○ Neutral
○ Angry

Given the emotion you selected, how would you rank the degree of that emotion expressed by this individual?

Very Low	Low	Medium	High	Very High
○	○	○	○	○

Figure 6. Rating instructions and a sample rating trial as seen in the online survey. For each stimulus, participants were asked to identify the emotion displayed as well as the degree of that emotion (Very Low to Very High).

Table 2

Valence Values for Face Stimuli as Scored by SONA Participants

Familiar				Unfamiliar			
Model	Negative	Neutral	Positive	Model	Negative	Neutral	Positive
Adam(1)	-1.03 +/- 0.14	-0.90 +/- 0.13	3.47 +/- 0.09	Adam(2)	-2.22 +/- 0.12	-0.60 +/- 0.11	2.46 +/- 0.11
Ashton	-2.76 +/- 0.11	-0.16 +/- 0.08	3.39 +/- 0.10	Alex	-2.67 +/- 0.16	-0.20 +/- 0.08	4.68 +/- 0.08
Fanie	-1.18 +/- 0.25	-0.36 +/- 0.11	3.83 +/- 0.08	Jami	-2.01 +/- 0.16	-0.75 +/- 0.14	4.47 +/- 0.07
Lizzie	-1.60 +/- 0.22	-0.05 +/- 0.10	4.49 +/- 0.11	Janice	-3.20 +/- 0.10	-0.94 +/- 0.13	3.76 +/- 0.08
Melanie	-4.24 +/- 0.09	-0.23 +/- 0.08	2.69 +/- 0.12	Martha	-2.52 +/- 0.12	0.44 +/- 0.10	3.90 +/- 0.08
Michael	-0.15 +/- 0.07	0.35 +/- 0.13	3.61 +/- 0.12	Megan	-2.75 +/- 0.12	-0.26 +/- 0.10	3.76 +/- 0.11
Paul	-3.00 +/- 0.10	-0.46 +/- 0.12	3.05 +/- 0.12	Steven	-2.00 +/- 0.16	-0.18 +/- 0.07	3.99 +/- 0.09
Rose	-1.80 +/- 0.14	0.07 +/- 0.05	3.64 +/- 0.12	Terry	-3.24 +/- 0.14	-1.89 +/- 0.16	3.55 +/- 0.09
Gigi	1.54 +/- 0.14	-0.60 +/- 0.13	4.85 +/- 0.04				

Experiment 1b: Visual fMRI Task

Dog were presented with familiar and unfamiliar faces expressing positive, neutral, and negative emotions (as developed in Experiment 1a) in the functional imaging experiment. The purpose of this experiment was to uncover neural activation patterns mediated by familiarity and emotion. The findings will be used to develop methods for identifying successful working dogs via neural and behavioral patterns. The visual task was a passive one in which dogs were presented with stimuli via an in-scanner projector screen. Although this task did not provide a direct behavioral measure, we know from past research that dogs can discriminate between faces expressing different emotional content (Müller, Schmitt, & Huber, 2015). To investigate the neural mechanisms of dogs' sensitivity to faces and emotions, we targeted differential activation patterns that were mediated by familiarity and emotional valence of stimuli. We hypothesized that activation patterns would be analogous to those found in humans and non-human primates, notably in the amygdala and hippocampus. Further, we anticipated similar face- and familiarity-mediated activations to those found in past dog fMRI studies, such as those in the caudate and temporal regions.

Method

Subjects

We used a cohort of 40 working dogs in this study. The cohort participated in visual task runs and later participated in behavior measures (see Experiment 2). All dogs were procured and trained by iK9, LLC and included Labrador Retrievers, Springer Spaniels, Belgian Malinois, and German Shepherds. All dogs were between 6 months and 3 years of age. Both male and female dogs were used in training and in scanning. All dogs remained conscious for imaging, for which they were trained to lie in a prone position on the scanner bed with head inserted into a human knee coil. Positive reinforcement was provided to keep dogs as still as possible and to desensitize them to the scanner environment. Ethical approval for this study was obtained from the Auburn University Institutional Animal Care and Use Committee and all methods were performed in accordance with their guidelines and regulations.

Training

In order for dogs to lie motionless and awake while unrestrained in the scanner, progressive positive-reinforcement training was implemented. Training progressed from basic behavioral shaping using the clicker/treat and target stick methods, through off-site mock scanner training, and finally to training in the scanner environment. Clicker training involves the pairing of a food reward with a “click” in order to create a marker for appropriate behavior. In early training, the appropriate behavior of touching the snout to a target stick was rewarded. Clicks and treats were presented at a rapid rate (e.g. every 2 seconds as long as the desired behavior was maintained) and this time span gradually increased until a dog maintained the appropriate behavior for several minutes. The use of a target stick ensured appropriate positioning of the dog in the scanner. In the functional imaging experiment, the appropriate behavior was defined as lying motionless in the prone position with his/her head in the coil for three to five minutes.

Clicker and treat training were conducted, along with scanner audio acclimation, in the mock scanner (Figure 7) until the dog demonstrated ceiling performance. The dogs then entered MRI suite acclimation training, wherein they were first allowed to adjust to the sights and sounds of the scanner environment by walking around the suite and climbing onto the patient table. When the dog demonstrated ease in the scanner room, clicker and treat training were reintroduced inside the scanner (Figure 8). When the dog again reached the appropriate behavior criterion, he/she was deemed ready for the experiment proper.

Figure 7. The mock coil used in pre-scanner training. Dogs were trained to lie motionless and awake while unrestrained with the aid of clicker/treat training. An audio recording (CD) of scanner noise was played at increasing sound levels to acclimate the dog to the environment.

Figure 8. Transitional training was conducted in the MRI suite to further acclimate the dogs to the MR environment in preparation for scanning.

Experimental Design

The setup comprised of 3T Siemens Verio scanner, the human knee coil adapted as a dog head coil, a projector system to present visual stimulus and an external infra-red camera used to track head motion in dogs and retrospectively correct for motion artifacts in the data. Functional data was obtained from the 3T Siemens Verio scanner using an EPI sequence with the following parameters: repetition time (TR)=1000 ms, echo time (TE)=29 ms, field of view (FOV)=192×192 mm^2, flip angle (FA)=90 degree, in-plane resolution 3×3 mm, in-plane matrix 64×64, and whole brain coverage. Anatomical data was obtained for registration purposes using an MPRAGE sequence with the following parameters: TR=1550 ms, TE=2.64 ms, voxel size: 0.792×0.792×1 mm^3, FA=9°, in-plane matrix = 192×192, FOV=152×152 mm^2, number of slices: 104.

During scanning sessions, each dog completed four runs of randomized order, including two runs of images and two runs of videos. Each run totaled 140 seconds and included either 12 stimuli (human faces only) or 20 stimuli (human and dog faces). Stimuli were presented via projector screen for five seconds, after which a blank screen was presented for a variable 3- to 11-second inter-stimulus interval (ISI). A one second repetition time (TR) was used.

Stimuli. Both still images and videos were presented during scanning. Still images consisted of familiar and unfamiliar human faces displaying positive, neutral, and negative emotions. For a subset of dogs (N = 12), still images of dog faces were also included. Videos consisted of 5-second clips of familiar and unfamiliar human faces displaying positive, neutral, and negative emotions. For positive videos, humans stated, “Good dog” repeatedly in a jubilant tone. For neutral videos, humans repeated “We’re gonna do this, we’re gonna do that,” with lack of affect. For negative videos, humans repeated “Bad dog” in forceful tone.

Attention Scoring

To be sure that each dog looked at each stimulus that was presented during scanning several precautions were taken. Such precautions were necessary to assure that only trials in which the dogs attended to the stimulus were analyzed. Attention was judged by multiple raters via simultaneous video recording of stimulus presentation and the dog's eye (Figure 9). For each trial, if the dog's eye was visibly open, then the rater assigned a score of "yes". If the dog's eye was closed or not open enough that the pupil was visible, then the rater assigned a score of "no". The form used for scoring is seen in Figure 10. Inter-rater reliability was assessed for each trial, and trials with inter-rater agreement of attentiveness were retained for data analysis.

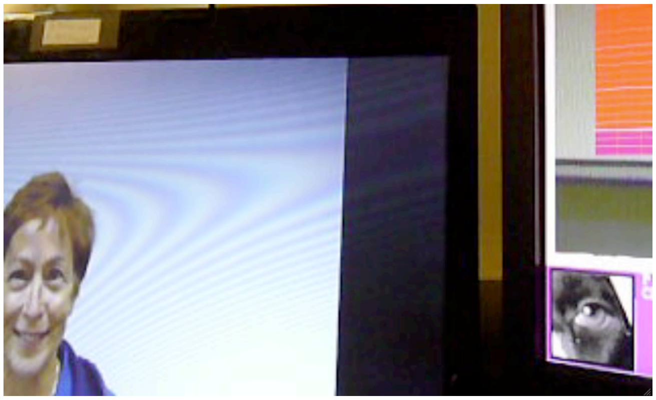
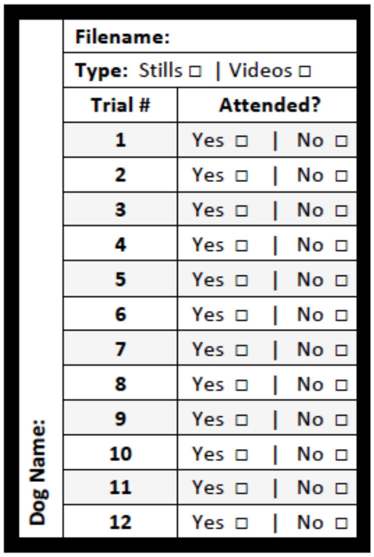


Figure 9. A screenshot of a video recording used for attention scoring. The stimulus presentation is shown on the left side of the screen and the eye-tracker capture of the dog's eye is shown on the right side of the screen. Timing for each component was simultaneous.



Dog Name:

Filename:

Type: Stills ☐ | Videos ☐

Trial #	Attended?	
1	Yes ☐	No ☐
2	Yes ☐	No ☐
3	Yes ☐	No ☐
4	Yes ☐	No ☐
5	Yes ☐	No ☐
6	Yes ☐	No ☐
7	Yes ☐	No ☐
8	Yes ☐	No ☐
9	Yes ☐	No ☐
10	Yes ☐	No ☐
11	Yes ☐	No ☐
12	Yes ☐	No ☐

Figure 10. Scoring form for each fMRI run used by attention scorers.

Data Retention

Of forty dogs, 37 had usable fMRI data for both still image and video tasks. Data for 9 dogs in the still images task and 11 dogs in the videos task were eliminated due to excessive motion. Data was insufficient for contrasts for 7 dogs in the images task and 6 dogs in the videos task. In all, 48 still image runs were retained for a total of 21 subjects. Of the images presented, more than 80% were rated as seen by attention scorers. Forty-nine video runs were retained for a total of 20 subjects. Of the videos presented, more than 80% were rated as seen by attention scorers. Count and percentage breakdowns for still images and videos by condition are shown in Table 3.

Table 3

Counts and percentages of images attended to during still image and video runs.

	Still Images		Videos	
Condition	Count	Percentage	Count	Percentage
Familiar	242/288	84.02%	244/294	82.99%
Unfamiliar	246/288	85.41%	247/294	84.01%
Positive	164/192	85.41%	169/196	86.22%
Neutral	158/192	82.29%	159/196	81.12%
Negative	166/192	86.45%	163/196	83.16%
Total	488/576	85.31%	491/588	83.50%

Image Processing

Data processing was conducted using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/, Functional Imaging Lab, The Welcome Trust Centre for NeuroImaging, The Institute of Neurology at University College London). All data were run through standard preprocessing steps, including realignment to the first functional image, spatial normalization to a template, and spatial smoothing (discussed in Jia et al., 2014). Following preprocessing, the general linear model (GLM) was applied and statistical tests revealed voxels which were activated for each condition comparison. The first level of analysis involved individual subject data. T-tests were run with familiar face stimuli against unfamiliar face stimuli and positive (happy) face stimuli against both neutral and negative (angry) faces. A threshold of $p < 0.05$ was used. Next, second level group analyses were conducted for familiar faces against unfamiliar faces and positive (happy) faces against both neutral and negative (angry) faces. Significant areas of activation were identified in the caudate, hippocampus, and amygdala for still image and video presentations.

Results

Image Presentations

Mean beta weights and standard deviations are reported. For image presentations, caudate activation was revealed for familiar versus unfamiliar faces (M = 2.30, SD = 4.76), positive versus neutral faces (M = 3.01, SD = 3.24), and negative versus neutral faces (M = 2.10, SD = 2.55). Right hippocampus activation was also seen for the negative versus neutral contrast (M = 1.89, SD = 2.25). Figure 11 shows the identified regions of activation characteristic of the still images task. Warmer colors reflect higher beta weights.

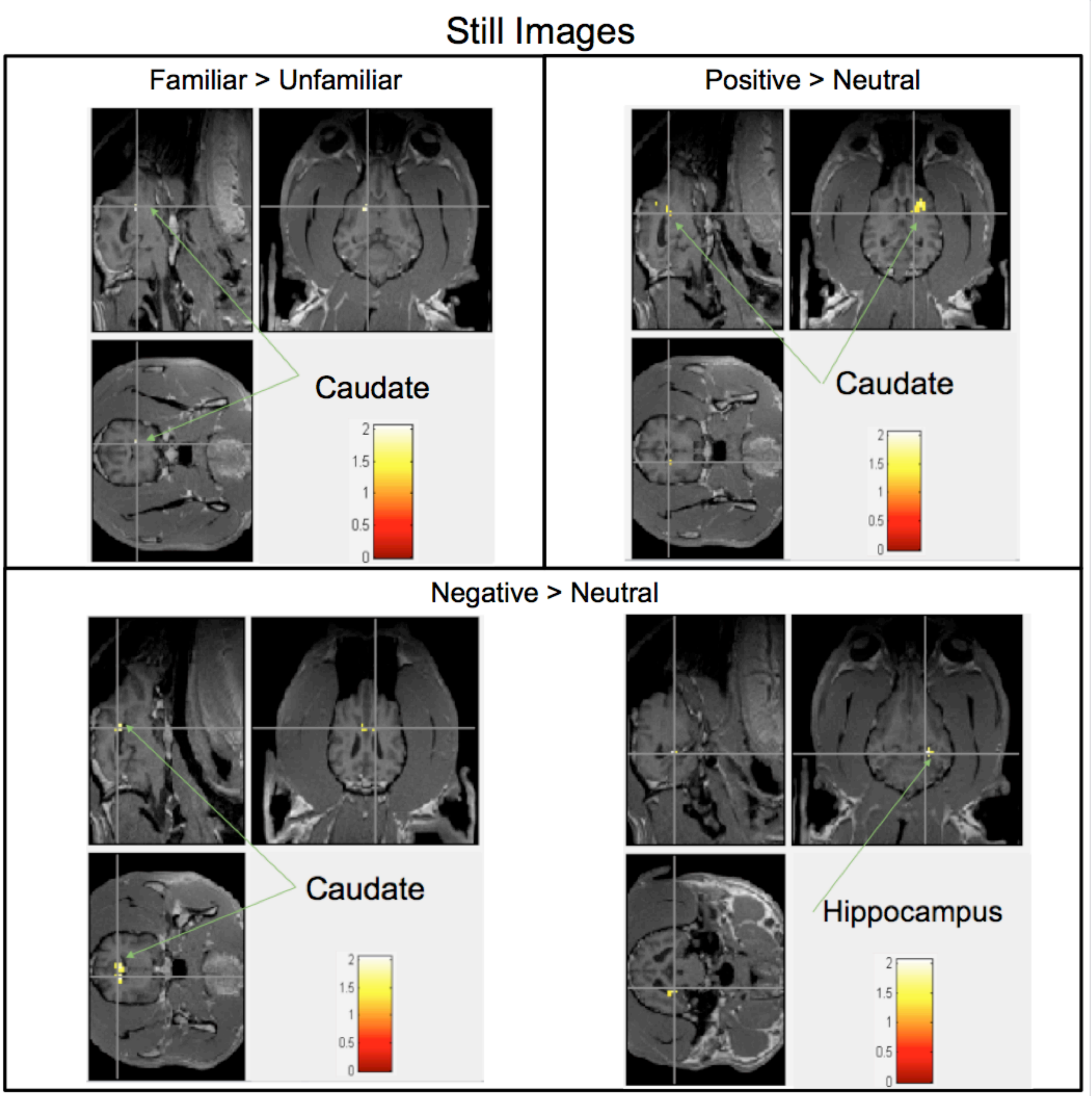


Figure 11. Activation maps for images. Three orthogonal views are shown for each subfigure. A color map is used for activation intensity, with warmer colors corresponding to higher beta values.

Video Presentations

Mean beta weights and standard deviations are reported. For video presentations, caudate activation was revealed for familiar versus unfamiliar faces (M = 2.61, SD = 5.32) and negative versus neutral faces (M = 2.93, SD = 3.09). Left amygdala activation was shown for familiar versus unfamiliar faces (M = 6.41, SD = 13.45), positive versus neutral faces (M = 5.59, SD = 7.17) and negative versus neutral faces (M = 3.95, SD = 4.01). The positive versus neutral face contrast also revealed left hippocampus activation (M = 3.80, SD = 6.40). Figure 12 shows the identified regions of activation characteristic of the videos task. Warmer colors reflect higher test statistics.

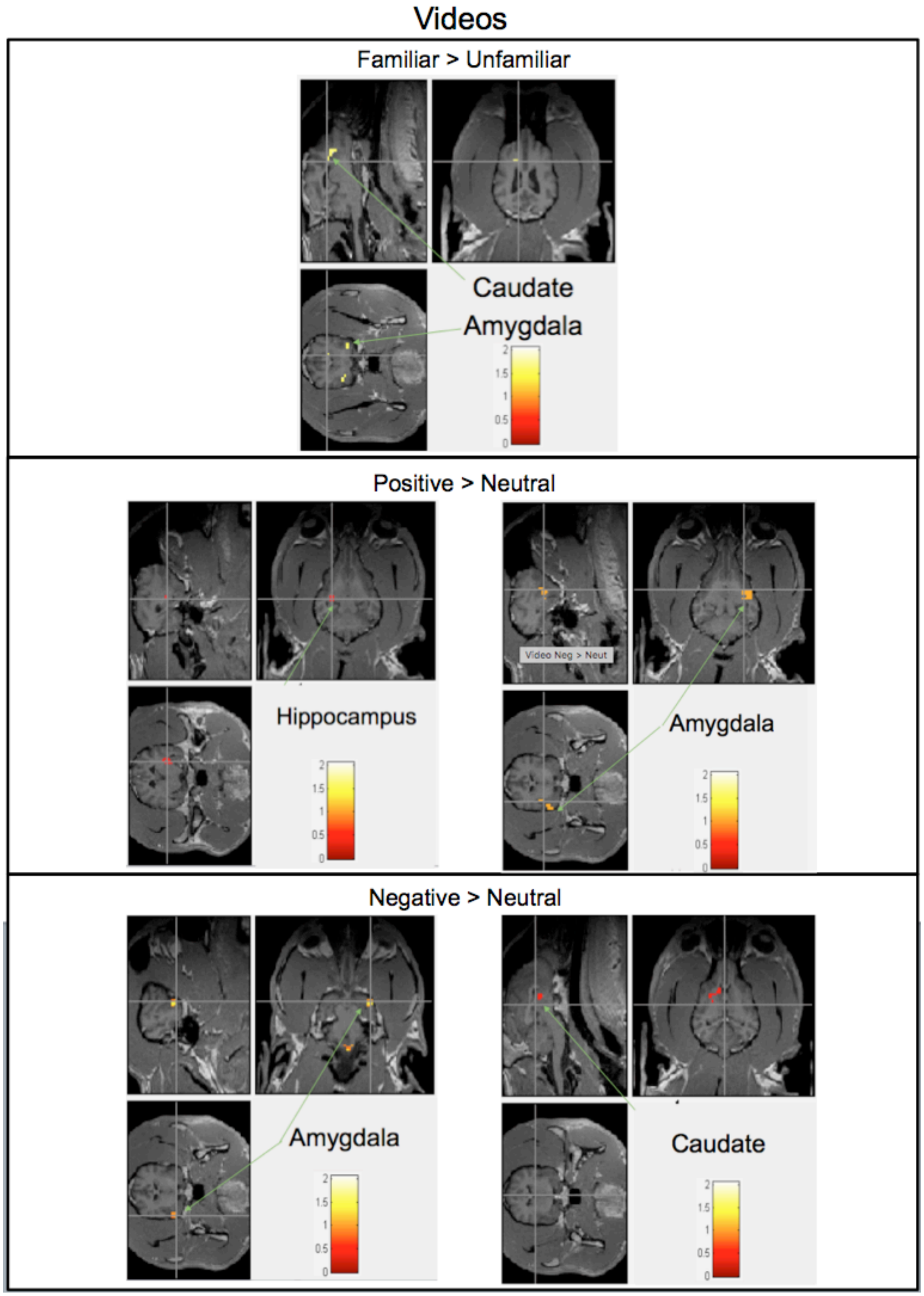


Figure 12. Activation maps for videos. Three orthogonal views are shown for each subfigure. A color map is used for activation intensity, with warmer colors corresponding to higher beta values.

Human Faces versus Dog Faces. In order to investigate the effects intra- and interspecific face recognition, T-values were used to identify regions of the brain that were differentially active for a subset of dogs that had been exposed to both stimulus types in the still images task. This contrast revealed differences in activation localization for human faces and dog faces in the left temporal lobe (Figure 13).

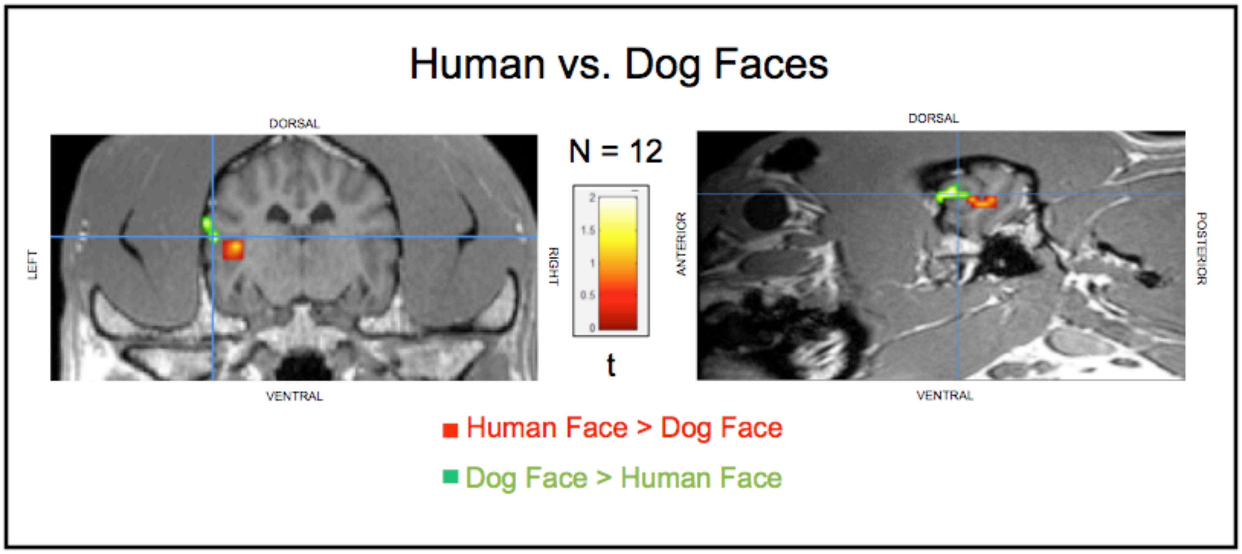


Figure 13. Activation maps showing differential activation in the temporal lobe mediated by species. A color map is used for activation intensity, with warmer colors corresponding to higher beta values.

Discussion

Experiment 1 was designed to develop and implement a visual stimulus set that was socially relevant to a targeted working dog sample. The stimulus was then used to investigate mediation of neural activation in dogs by familiarity and emotional valence. It was hypothesized that when presented with stimulus sets bearing conditions of familiar versus unfamiliar faces and positive, neutral, and negative emotion valences, dogs' neural activation patterns would vary according to these condition contexts. This hypothesis was supported as differential activation patterns were uncovered for familiar versus unfamiliar faces, positive versus neutral faces, and negative versus neutral faces. Differential activations mediated by familiarity were identified in the caudate and left amygdala. Differential activations mediated by emotion valence were identified in the caudate (familiar, positive, and negative images; familiar and negative videos), left amygdala (familiar, positive, and negative videos), and hippocampus (negative images; positive videos). Additionally, separate but adjacent areas of activation for human and dog faces were identified. These findings are explored further in the general discussion.

Experiment 2: The Unsolvable Task

The unsolvable task was appropriate for the current research due to its efficacy in revealing familiarity biases in a range of dog populations. Here, we sought to parse out behavioral tendencies to seek the assistance of a familiar person and/or demonstrate attachment to a familiar person during stressful situations. The working dog population that was used for this study (primarily odor detection dogs) was unique in that these dogs are trained to focus on environmental cues rather than collaboration with humans. However, although these dogs are relatively more independent than pet dogs, it could be expected that when turning to a human for help the familiar handler would be a more likely choice.

Method

Subjects

The sample for this experiment consisted of the same dogs used in the visual fMRI task. These dogs were trained, handled, and cared for by iK9. Ethical approval was obtained from the

Auburn University Institutional Animal Care and Use Committee and all methods were performed in accordance with their guidelines and regulations.

Apparatus

The apparatus (Figure 14) was constructed of a plywood base (26" x 20" x 1.5"), upon which the lid to a Sterilite 2.5-qt ($7^3/_8$" x $5^5/_8$" x 6") storage container was mounted upside down. The container could then be placed upside down on the lid to conceal a treat (Purina Moist and Meaty pellet) or the dog's toy. If the container was left unlocked, then the dog had easy access to the treat/toy during solvable trials (nose-poke pressure was enough to knock the container off the lid). If the container was locked, the dog was unable to access the treat or toy.

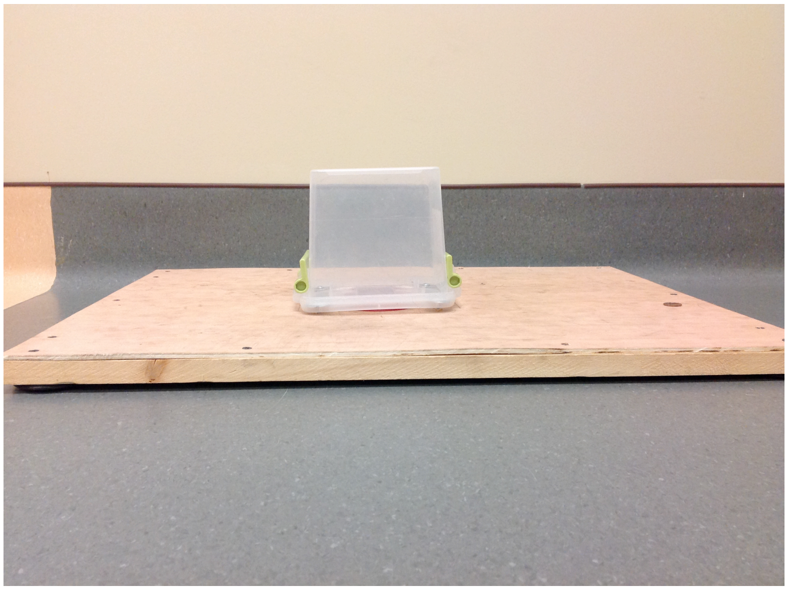


Figure 14. The apparatus used in the unsolvable task is seen here in the unlocked (solvable) position. In the unsolvable condition, the green handles were lowered and locked onto the container lid.

Experimental Design

The unsolvable task conducted in this research was comprised of a four-person experimental team. The experimenter organized and set up task trials and recorded session information. The dog's trainer served as the familiar human and an undergraduate research assistant served as the unfamiliar human. The assisting researcher handled the dog during experimental sessions.

The task was characterized by the familiarity of human models and the accessibility of the treat or toy. The unfamiliar human was defined as a research assistant that did not work with the dog on a regular basis and did not have a history of giving the dog commands and/or rewards. In contrast, the familiar human was defined as the owner/trainer who regularly interacted with, cared for, and/or conducted training with the dog. Accessibility of the treat or toy was defined by the trial condition. During solvable trials, the dog was able to access the reward within the apparatus. During unsolvable trials, the apparatus was locked and the dog could not access the reward.

Procedure

An acclimation period was allowed before each session began. During this time, the dog was monitored and allowed to roam until he/she became visibly comfortable in the testing room. Stress indicators (panting, whining, etc.) were assessed and if such indicators were absent after 5 minutes, the dog was cleared to begin pre-training.

Each experimental session was preceded by pre-training. A series of demonstration trials were given in order to establish that manipulation of the apparatus resulted in a treat reward. That is, dogs were shown that the apparatus could be knocked over to reveal a reward and the dog was gradually trained to knock the apparatus over on his/her own. Prior to each demonstration, the handler brought the dog into the room and held him/her by the collar until the demonstrator gave the signal to release the dog. Once the dog reliably approached and knocked over the barrier to reveal the reward, the experimental session began.

The experimental schematic is depicted in Figure 15. To begin a trial, the handler brought the dog into the training/testing area. The familiar human stood at his/her designated task position with head forward and the unfamiliar human stood at his/her analogous (mirrored) task position. When the dog was positioned appropriately at the starting point, the experimenter said, “Okay” and the handler released the dog. The dog was given 15 seconds to interact with the apparatus. However, the trial was marked as complete when the dog obtained all of the treat reward(s) in the solvable condition or when the dog had diverted his/her attention from the apparatus for more than 15 seconds. Unsolvable trials were continued for 15 seconds. Each trial was separated by a 30-s inter-trial interval (ITI), during which the dog was removed from the arena.

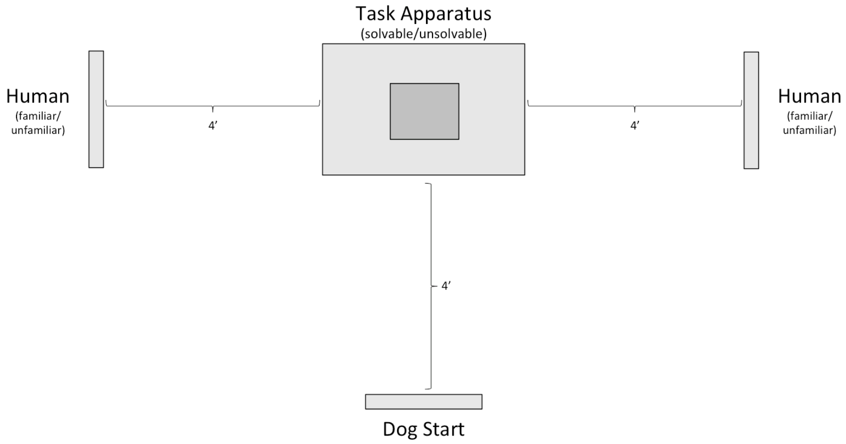


Figure 15. The testing arena shows dog position in front of the apparatus and human positions to the left and right (counterbalanced). The apparatus was unlocked during solvable trials and locked during unsolvable trials.

Post-Session Coding

Each session was videotaped and later coded by two or more researchers and/or research assistants. The scoring sheet (Appendix A) was used to tally showing behaviors (Figure 16) to the familiar and unfamiliar person, as well as nonspecific behaviors such as barking or targeting (e.g. staring at) the apparatus. Video time points and behavior durations were also reported. These coding worksheets were later put into Microsoft Excel format and organized for data analysis.

Figure 16. A subject demonstrating a showing behavior during an unsolvable trial.

Results

Data for 27 dogs were obtained. Behavioral data was organized in Microsoft Excel and analyzed using SPSS software. Results of the unsolvable task were analyzed to uncover individual- and cross-population differences in showing behaviors made toward familiar versus unfamiliar humans. Data were grouped by subject, trial condition (solvable/unsolvable), and response type (unfamiliar/familiar/nonspecific) as factors. It was hypothesized that, across populations, dogs would seek help from their human companion more so than from an unfamiliar human. That is, we expected dogs to exhibit a greater frequency of attempts to engage the familiar human and that they will also spend longer amounts of time doing so.

In scoring unsolvable task data, both the number of times the dog demonstrated a showing behavior and the total amount of time they spent demonstrating those behaviors were counted. The data were coded using three categories of showing behaviors: behaviors directed at the familiar person, behaviors directed at the unfamiliar person, and nonspecific showing behaviors. Showing behaviors include looking, barking, pawing, sitting, jumping, and gazing. Each data set consisted of four solvable and four unsolvable trials.

Figure 17 illustrates trial progression and shows trial duration was mediated by solvability of the task. The first four trials were solvable and trial duration was consistent (M = 4.95, SD = 2.78), as confirmed by a one-way repeated-measures ANOVA over trials (1,2,3,4), $F(3,81) = 1.93$, $p = 0.13$. The last four trials were unsolvable and trial duration was the maximum time allowed. Data were further broken out into frequencies and durations of showing behaviors.

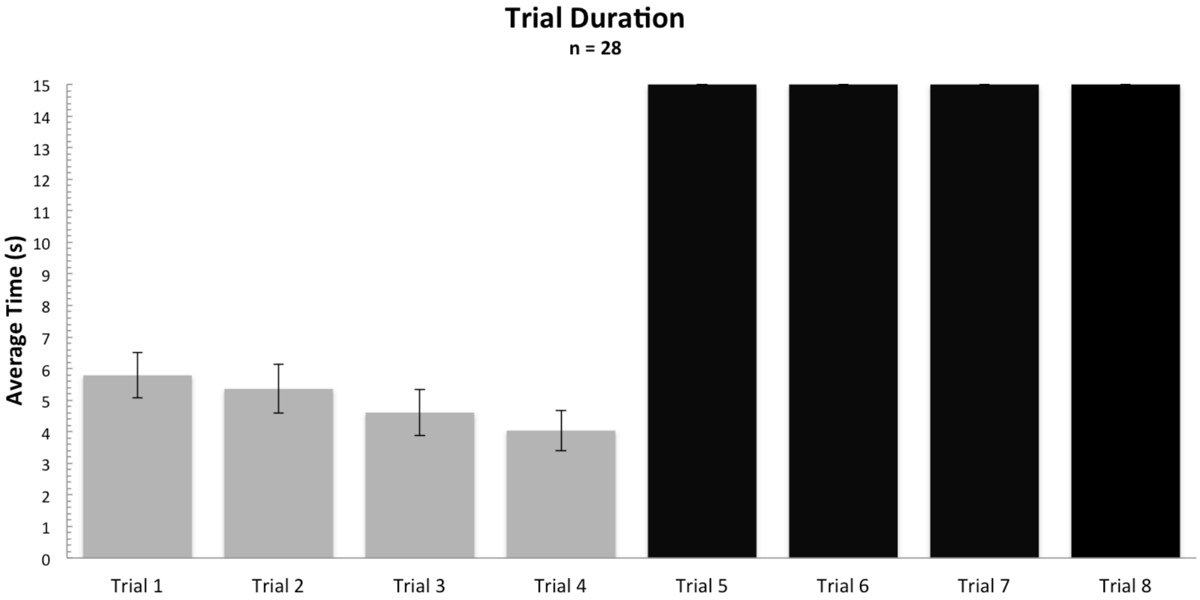


Figure 17. Total time to trial completion for solvable (1 to 4) and unsolvable (5 to 8) trials in the unsolvable task.

Figure 18 shows frequencies of showing behaviors across trials (1 to 8) and direction of behavior (familiar person, unfamiliar person). Trials 1 through 4 were solvable and yielded fewer instances of showing than did trials 5 through 8, during which the task was unsolvable. To confirm that performance was stable over trials, a series of one-way repeated-measures ANOVAs were conducted: solvable trials (familiar: F (3, 81) = 0.49, p = 0.69; unfamiliar: F (3, 81) = 1.35, p = 0.26) and unsolvable trials (familiar: F (3, 81) = 1.49, p = 0.22; unfamiliar: F (3, 81) = 2.09, p = 0.11). A two-way repeated measures ANOVA with familiarity (familiarity, unfamiliar) and solvability (solvable, unsolvable) as factors on showing behavior frequencies revealed a main effect of solvability, $F(1, 27) = 31.29$, $p < 0.01$, $\eta_p^2 = 0.54$, but no effect of familiarity, F (1, 27) = 2.91, p = 0.10. There were no subject effects or interactions, F (1, 27) = 0.97, p = 0.33.

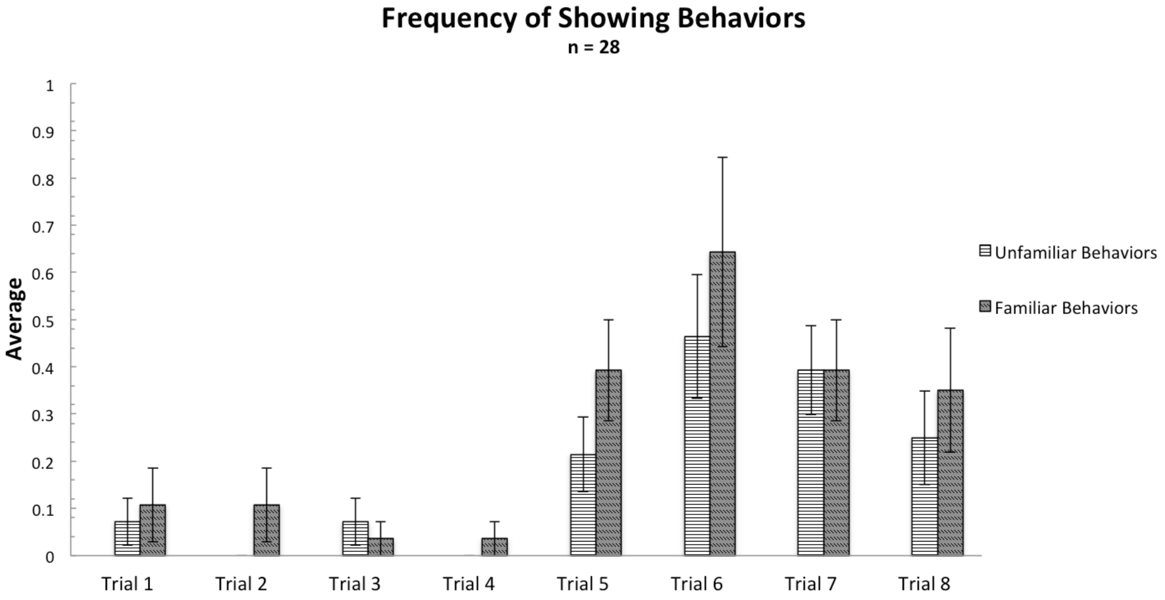


Figure 18. Frequency of showing behaviors in the unsolvable task grouped by direction toward familiar or unfamiliar model. Frequencies are separated by trial, with solvable trials occurring from 1 to 4 and unsolvable trials occurring from 5 to 8.

Figure 19 shows durations (in seconds) of showing behaviors as grouped by trial (1 to 8) and direction of behavior (familiar person, unfamiliar person). Trials 1 through 4 were solvable and yielded shorter durations of showing behaviors than did trials 5 through 8, during which the task was unsolvable. Durations were longer for unsolvable trials than for solvable trials. Across solvable and unsolvable trials, durations of showing behaviors were greater for familiar individuals. Trial stability was confirmed by one-way repeated-measures ANOVAs for solvable trials (familiar: F (3, 81) = 0.72, p = 0.54; unfamiliar: F (3, 81) = 1.35, p = 0.54) and unsolvable trials (familiar: F (3, 81) = 1.14, p = 0.34; unfamiliar: F (3, 81) = 2.67, p = 0.05). A two-way repeated measures ANOVA with familiarity (familiarity, unfamiliar) and solvability (solvable, unsolvable) as factors on showing behavior durations revealed main effects of both familiarity,

$F(1, 27) = 5.38$, $p < 0.05$, $\eta_p^2 = 0.17$), and solvability, $F(1, 27) = 26.33$, $p < 0.01$, $\eta_p^2 = 0.49$). There were no subject effects or interactions, $F(1, 27) = 3.00$, $p = 0.09$.

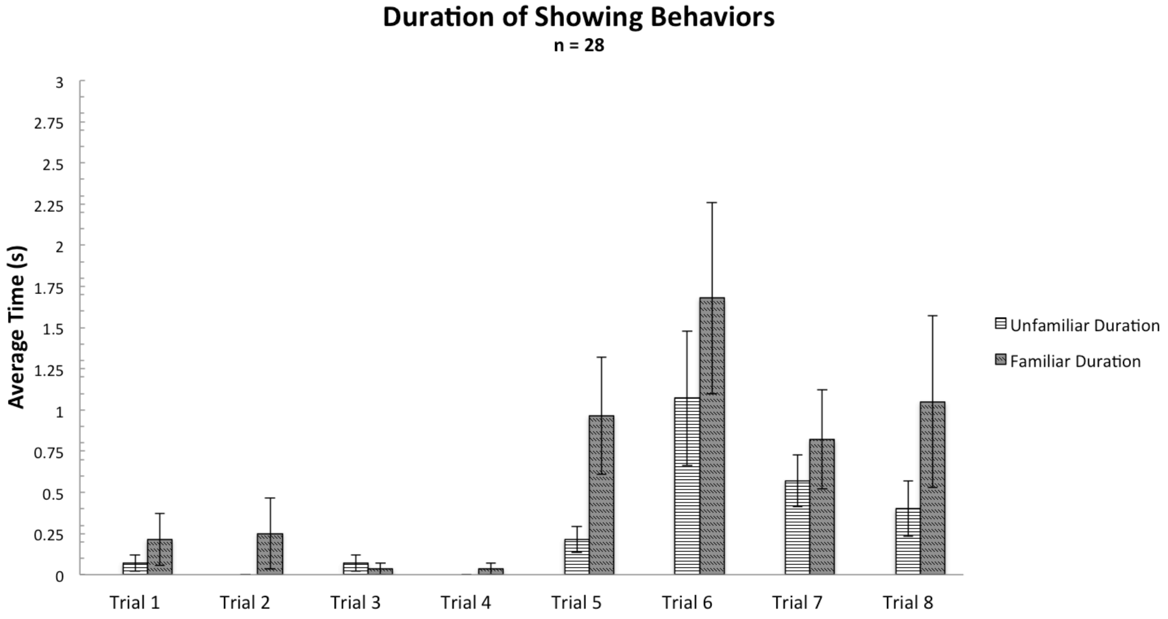


Figure 19. Duration of showing behaviors in the unsolvable task grouped by direction toward familiar or unfamiliar model. Durations are separated by trial, with solvable trials occurring from 1 to 4 and unsolvable trials occurring from 5 to 8.

Final Scores for Correlation

Composite bias scores for frequency and duration were calculated by subtracting behaviors to the unfamiliar person from behaviors to the familiar person. Thus, positive values indicate a bias toward the familiar person, and negative values indicate a bias toward the unfamiliar person. Frequency scores (N = 28) ranged from -2 to 4 (M = 0.43, SD = 1.55). Duration scores (N = 28) ranged from -8 to 16 (M = 2.10, SD = 5.30). Scores for each dog can be seen in Table 4. These single-point scores were used for correlation with neural data.

Table 4

Final Scores for the Unsolvable Task

Familiar		
Dog	Frequency	Duration
Blair	0	0
Blaine	1	6
Evie	-2	-3
Ikia	1	1
Pierce	4	8
Roxy	3	14
Violet	-1	-2
Sam	0	0
Envy	-2	-1
Fanie	-1	3
Sissy	1	1
Demi	0	7
Hannah	1	1
Ferris	3	16
Alisa	-1	-1
Daisy	0	-8
Guci	1	1
Hutch	1	10
Blue	2	2
Argo	-1	0
Branson	0	0
Blondie	1	0
Elerbe	-1	-3
Daffy	-2	-3
Mere	2	8
Mona	2	2
Mickey	0	0
Tazzy	0	0

Imaging Data Correlated with Behavioral Data

A primary aim of the current research was to reveal neural markers of attachment tendencies in the domestic dog. Composite scores of performance in the unsolvable task were correlated with visual task activation data on an individual dog basis. That is, a final familiarity bias score was calculated for each dog by subtracting the total duration of showing behaviors to the unfamiliar person during unsolvable trials from the duration of those made to the familiar person. These values were correlated with t-values of familiar versus unfamiliar activation and emotional valence activations. If neural activation is indicative of attachment as displayed in behavioral tests (and vice versa), then we could expect to find a relationship between each dog's activation and unsolvable task data.

Results

Preprocessing and Analysis. Data processing was conducted using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/, Functional Imaging Lab, The Welcome Trust Centre for NeuroImaging, The Institute of Neurology at University College London). All data were run through standard preprocessing steps, including realignment to the first functional image, spatial normalization to a template, and spatial smoothing. For each voxel, T-map values for all subjects were correlated with each subject's corresponding behavioral scores. Voxels for which a significant correlation ($p < 0.05$) was observed were retained.

Correlations. Sixteen dogs had usable data in both the fMRI and behavioral tasks. Correlational tests were run for still images and duration scores, still images and frequency scores, videos and duration scores, and videos and frequency scores. No significant correlations were found for frequency scores and the video task. Correlation of neural activations from the visual task with behavioral scores from the unsolvable task revealed significant correlation coefficients for areas in the amygdala, hippocampus, and caudate. Figures 20, 21, and 22 demonstrate the correlations between behavioral scores and the fMRI tasks.

Duration scores correlated with still image task. For familiar versus unfamiliar faces, significant correlations were found in the left and right amygdala (r = 0.50), left hippocampus (r = 0.59), and caudate (r = 0.67). For positive versus neutral faces, a significant correlation was shown in the left hippocampus (r = 0.58).

Duration scores correlated with videos task. For familiar versus unfamiliar face videos, significant correlations were found in the left and right amygdala (r = 0.74), caudate (r = 0.64), and left and right hippocampus (r = 0.62).

Frequency scores correlated with still image task. For familiar versus unfamiliar face images, significant correlations were found in the left hippocampus (r = 0.79) and left and right amygdala (r = 0.74). For negative versus neutral faces, significant correlations were found in the left and right hippocampus (r = 0.67), left and right amygdala (r = 0.61), and caudate (r = 0.52). For positive versus neutral faces, significant correlations were found in the left amygdala (r = 0.75) and left hippocampus (r = 0.67).

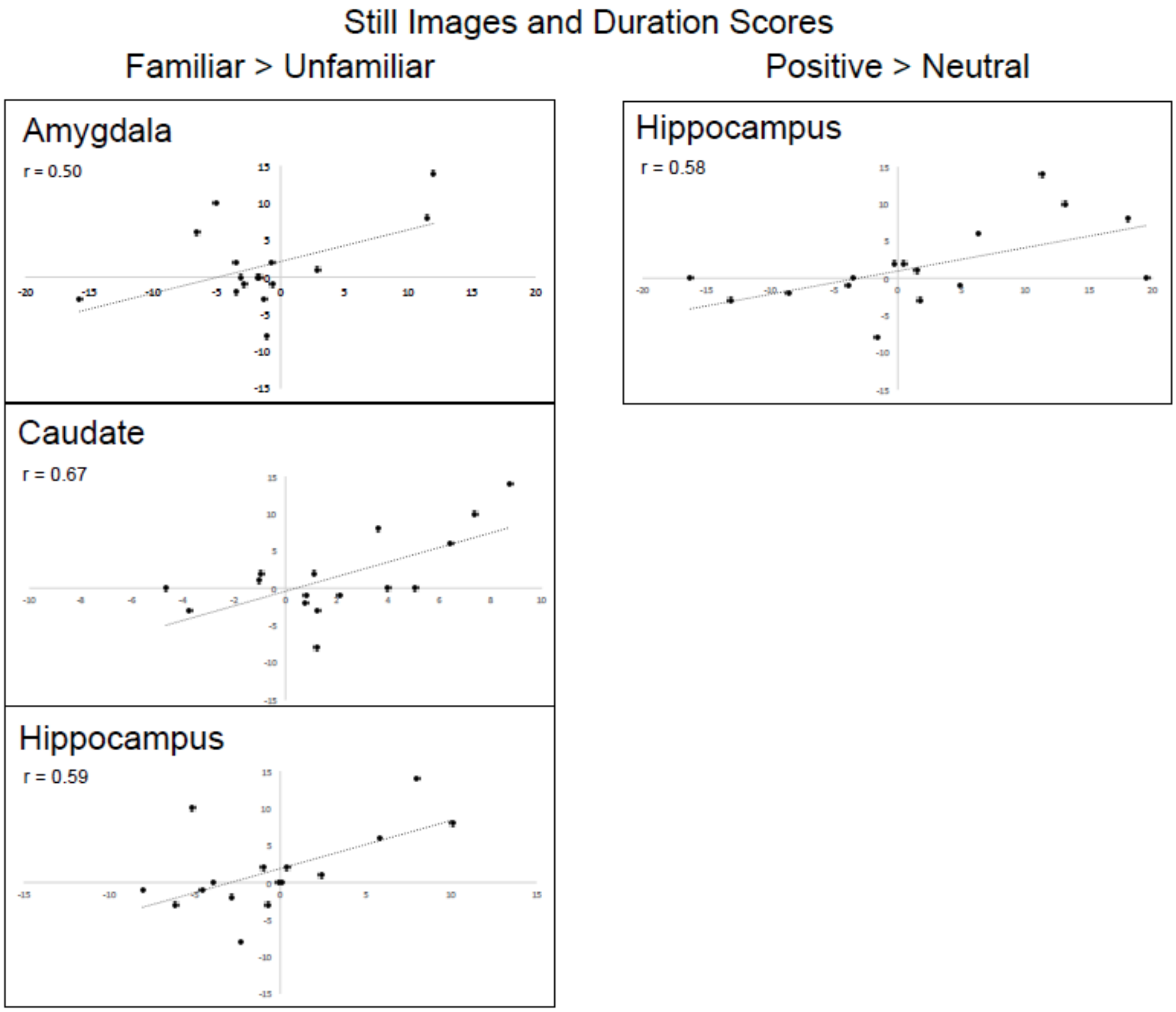


Figure 20. Duration scores correlated with the still images task. Voxel-wise correlation yielded the amygdala, caudate, and hippocampus as areas of significant correlation.

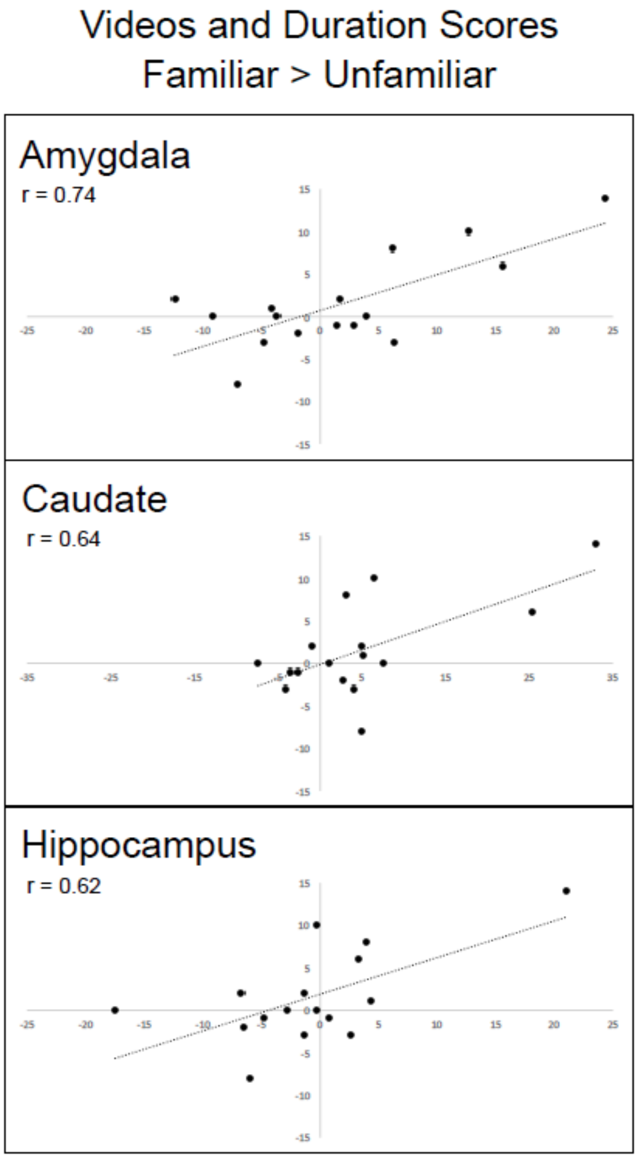


Figure 21. Duration scores correlated with the videos task. Voxel-wise correlation yielded the amygdala, caudate, and hippocampus as areas of significant correlation.

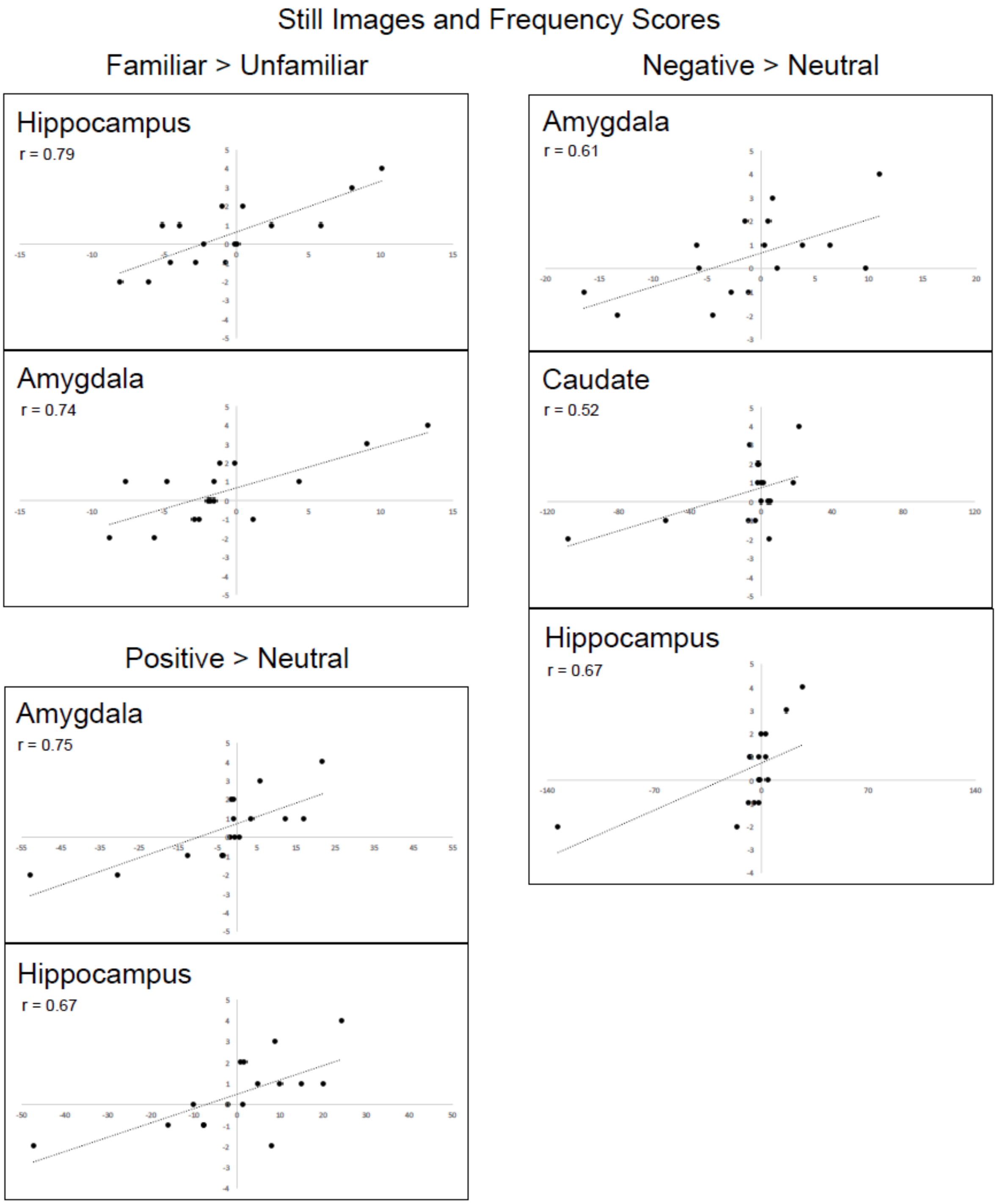


Figure 22. Frequency scores correlated with the still images task. Voxel-wise correlation yielded the amygdala, caudate, and hippocampus as areas of significant correlation.

Discussion

In this experiment, the unsolvable task was used to uncover biases toward familiar humans when encountering a problematic scenario. This task presents the dog with an easy behavior-outcome pattern of moving a barrier to obtain a reward. When this task becomes impossible, the dog may either persist or seek help in the environment. The behaviors emitted at this point, both in terms of frequency and duration, are of interest. When given the option of interacting with a familiar or unfamiliar person, past research has shown that dogs will more reliably approach familiar individuals (e.g. Virányi et al., 2012). The results found in this experiment are analogous to past findings, although there remains question as to whether duration of showing behaviors or frequency of showing behaviors is more valid for assessment of these biases.

The results indicate that even when the frequency of individual showing behaviors is stable across unfamiliar and familiar humans, the extent of these behaviors may be mediated by the level of familiarity. That is, when a dog has the opportunity to demonstrate a showing behavior toward a human with which he/she has a long repertoire of interaction, then he/she may persist longer in help-seeking. On the contrary, behaviors emitted toward the unfamiliar person may be due to novelty and environmental assessment, particularly when seeking new information as a previously achieved goal is being thwarted. Given this outcome, it appears that duration scores are more suitable for uncovering inherent familiarity biases than are frequency scores, as biased showing behavior is likely a difference of degree rather than occurrence.

In correlating the unsolvable task bias scores with neural data, several regions of interest were identified. When correlating with duration scores, significant areas included the caudate, amygdala, and hippocampus. When correlating with frequency scores, values for the caudate, amygdala, and hippocampus were again significant. Bio-behavioral correlations of the amygdala follow suit with familiarity preference, as this region is widely implicated in emotion and arousal across species and was further implicated in familiarity processing in the visual fMRI task presented here. Further, correlations of the hippocampus follow past human and non-human primate literature (e.g. Sliwa et al., 2014) as well as our hypotheses of familiarity recognition in the dog. The significance of the caudate in these results may be tied to the opportunity for command and reward as mediated familiarity of a human. Especially for the working dog population used in this study, heightened attention to a handler is imperative for learning and the receipt of commands and rewards. In sum, Experiment 2 provided behavioral evidence for familiarity preference, regardless of task solvability, in working dogs as well as a bio-behavioral index of familiarity preference when correlated with neural data from Experiment 1.

General Discussion

Experiment 1 investigated familiarity and emotional face processing in the domestic dog via still images and videos presented during fMRI. Experiment 1a served to develop the stimulus set to be used during scanning, consisting of still images of familiar and unfamiliar faces expressing positive, neutral, and negative expressions, as well as videos of familiar and unfamiliar humans expressing positive, neutral, and negative commands. Stimuli were scored by valence and the degree of the emotion displayed, yielding a score ranging from -5 (angriest) to +5 (happiest) for each stimulus. Final stimulus sets consisted of 24 images each, with 4 familiar and 4 unfamiliar individuals expressing the 3 different emotional expressions each.

Experiment 1b implemented in-scanner presentation of the stimuli that were developed in Experiment 1a. Forty dogs engaged in a passive viewing task after being trained to lie still during MR scanning. Each of 24 still images and 24 videos was presented for 5 seconds, varying along dimensions of familiarity (familiar/unfamiliar) and emotional content (positive, neutral, and negative). The data obtained in this experiment revealed differential activations in the caudate for familiar images, positive images, and negative images, as well as differential activation in the right hippocampus for negative images. Video presentations yielded differential activation in the caudate for familiar and negative videos, the right amygdala for familiar and positive videos, the left hippocampus for positive videos, and the right hippocampus for negative videos. Additionally, for a subset of dogs that were presented with both human faces and dog faces, adjacent areas of face processing in the left temporal lobe were revealed as mediated by species. A summary of activations is provided in Table 5.

Table 5

Summary of Activations for fMRI

Still Images			
Region	Familiar > Unfamiliar	Positive > Neutral	Negative > Neutral
Caudate	X	X	X
Amygdala	-	-	-
Hippocampus	-	-	X [right]

Videos			
Region	Familiar > Unfamiliar	Positive > Neutral	Negative > Neutral
Caudate	X	-	X
Amygdala	X [right]	X [right]	-
Hippocampus	-	X [left]	X [right]

The unsolvable task was conducted in Experiment 2. For the same dogs that were used in Experiment 1, a previously solvable task to obtain a food reward became impossible. Responses to familiar and unfamiliar individuals were recorded for frequency and duration. Data was obtained from 27 dogs and revealed effects of familiarity and solvability for duration of responding and an effect of solvability for frequency of responding. Final bias scores from the unsolvable task were correlated with neural data. Significant areas of correlation included the left amygdala, left hippocampus, and the caudate.

Comparisons to Past Face Processing Research. The findings from these experiments follow human and non-human primate literature and suggest similarity in processing of face stimuli across species. Paller et al. (2003) defines face processing as extraction of stimulus information, recall of face identity, and the extraction of cues used in socialization. With inclusion of variant levels of face familiarity and socially-relevant contextual information, the current research succinctly targeted each of these properties. Here we explore whether the findings of this research provide evidence for conservation of an earlier face processing system across species.

Haxby et al. (2000) outlined a model of human face processing that includes core processing in the occipital gyri, lateral fusiform gyrus, and superior central sulcus. In this model, an extended system of processing directs emotional content in the limbic system (e.g. amygdala). Stoeckel et al. (2014) directly explored human face processing by presenting scan participants with familiar dog (pet) faces and familiar human (child) faces seen by humans. Activations areas of particular interest were the amygdala, hippocampus, and fusiform gyrus. Extending the knowledge of face processing mechanisms to non-human primates, Sliwa et al. (2014) identified the hippocampus as active upon viewing familiar faces and Hadj-Bouziane et al. (2012) localized processing of emotional content on face stimuli to the amygdala.

In dogs, Dilks et al. (2015) presented dogs with movie clips of human faces, scenes, objects, and scrambled objects. Imaging data localized processing of dog and human faces to the inferior temporal cortex in the right hemisphere. Dilks et al. (2015) concluded that these activations represented the first evidence of a face-processing region in dogs. Later, Cuaya et al. (2016) presented dogs with stimuli of human faces and objects, identifying greater activations in the temporal cortex, frontal cortex, caudate nucleus, and thalamus for face stimuli.

The current experiment adds to this body of face processing research in that, like past human and non-human primate research, it targeted the role of face familiarity and emotional content in processing. As discussed, past investigations of familiar face processing have implicated the amygdala, hippocampus, and fusiform gyrus. For emotion processing, the amygdala has likewise been implicated. In the present study, we uncovered analogous face processing regions in the hippocampus and amygdala, and thus provide further evidence for a phylogenetically shared adaptation across species.

This research also adds to what is known about the mechanisms of face processing in domestic dogs and provides the first familiarity-based comparisons in this area of interest. Investigations of face processing in dogs have focused on human faces in opposition to inanimate and non-social content. Though familiarity has not previously been assessed, Dilks et al. (2015) and Cuaya et al. (2016) identified face processing areas in the temporal cortex and caudate. In the present study, we localized processing of familiar and emotional human faces to the hippocampus, amygdala, and caudate. Importantly, while hippocampal and amygdala activations may be stronger in our research due to stimulus emphasis on familiarity and emotion, the caudate, commonly referred to as the reward center, has been consistently implicated in face processing by dogs and highlights the social relevance of human face stimuli. In regards to temporal cortex activations, the findings shown here present separate but adjacent activation areas for presentations of human faces and dog faces. This contrasts with an overlapping region of activation indicated by Dilks et al. (2015), though this difference may be due to localization parameters.

Comparisons to Past Dog fMRI Familiarity Research. Although there have only been two published investigations of dog face processing (and no prior investigations of familiar or emotional face processing), imaging studies of familiar non-face content exist in the literature. Consistent with the visual domain, Cook et al. (2014) presented reward and no-reward hand signals as demonstrated by a familiar person or an unfamiliar person, as well as by digitized hand signal displays presented on a projection screen. Across the subject set, the caudate was differentially active by condition. Berns, Brooks, and Spivak (2015) investigated processing of socially-related stimuli via presentations of odors of familiar humans, unfamiliar humans, and dogs. Two regions of interest were revealed in olfactory bulb and the caudate, and the latter yielded differential activation according to odor type. For all dogs, the caudate was maximally activated for the odor of a familiar person. The findings of the current research fit well with past investigations of socially-relevant and familiarity-mediated content in the domestic dog. Here, we found consistent differential activations for familiar content in the caudate. This finding and its relation to past research highlights the relevance of human face stimuli to social cognition in the dog and further supports the notion of processing mechanisms that are shared across species.

Implications of Correlating Neural and Behavioral Data. Experiment 2 provided a unique opportunity to correlate neural and behavioral data within the same subject set. By drawing a tie between the social phenomenon of familiarity preference in-scanner via face presentation and out-of-scanner via the unsolvable task, questions of validity and applicability of dog fMRI may be explored. With either method alone, there is much to be desired in terms of final conclusions and translations across brain function and behavior. Due to the detrimental effects of in-scanner motion, behavioral responding is severely limited for the domestic dog. Whereas humans may use a mechanism such as a button box for behavioral assessment in fMRI, it is of yet unrealistic to plan and implement analogous response mechanisms for dogs. As such, replications of in-scanner processes of interest outside of the scanner offer the greatest opportunity for valid bio-behavioral conclusions in dog research.

Conclusions

The current study was developed to investigate the behavioral and neural indices of the dog-human social bond. This bond has been shaped by domestication over several thousands of years, and canine social cognition provides a rich avenue for research with dogs. The subsets of this field in which we were most interested were those that assess differential attention and behavior mediated by a dog's history and relationship with a particular human being. This area of behavior and cognitive processing is particularly relevant to working dogs, as their human handlers serve as both a companion and an instructor. The experiments presented herein targeted the attachment between dog and handler in a working dog population.

This research utilized a multi-method approach, merging the behavioral and neuroimaging avenues of investigation. We explored the neural processing of familiar faces and emotional expressions using fMRI. We also incorporated a well-documented behavioral assessment that targets dogs' attachment to their human partners. Simultaneous acquisition of behavioral and neural data allowed us to correlate findings so as to uncover potential profiles of successful working dogs.   In all, the hypotheses of the current research were supported. Hippocampus and amygdala activation appear to be mediated by both familiarity and emotional valence. We also found separate but adjacent activation areas for human faces and dog faces. Finally, we found that familiarity bias in a behavioral task correlates with amygdala activation in a neuroimaging task.

The results of this study will be expanded upon and complemented by relevant investigations, such as longitudinal timepoint shifts in responding and functional connectivity analyses. The field of canine neuroimaging is in its infancy, with less than 20 published investigations to date, and this project will contribute significantly to the foundations of this interest area. It is anticipated that this research will contribute to continued progress in the development of bio-behavioral profiles of successful working dogs.

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APPENDIX A

Scoring form for the unsolvable task

Unsolvable Task Score Sheet

Scored by: ____________________

Trial #1: Solvable/Unsolvable

#	Behavior Type	Start Time	Stop Time	Duration	Toward
1					
2					
3					
4					
5					
6					
7					

Trial #2: Solvable/Unsolvable

#	Behavior Type	Start Time	Stop Time	Duration	Toward
1					
2					
3					
4					
5					
6					
7					

Comments

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Dog: ____________________

Familiar: ____________________ Unfamiliar: ____________________

Trial #3: Solvable/Unsolvable

#	Behavior Type	Start Time	Stop Time	Duration	Toward
1					
2					
3					
4					
5					
6					
7					

Trial #4: Solvable/Unsolvable

#	Behavior Type	Start Time	Stop Time	Duration	Toward
1					
2					
3					
4					
5					
6					
7					

Comments

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Trial #5: Solvable/Unsolvable

#	Behavior Type	Start Time	Stop Time	Duration	Toward
1					
2					
3					
4					
5					
6					
7					
8					

Trial #6: Solvable/Unsolvable

#	Behavior Type	Start Time	Stop Time	Duration	Toward
1					
2					
3					
4					
5					
6					
7					
8					

Comments

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Trial #7: Solvable/Unsolvable

#	Behavior Type	Start Time	Stop Time	Duration	Toward
1					
2					
3					
4					
5					
6					
7					
8					

Trial #8: Solvable/Unsolvable

#	Behavior Type	Start Time	Stop Time	Duration	Toward
1					
2					
3					
4					
5					
6					
7					
8					

Comments

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