Neural Correlates of Emotion in Dogs

by

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Abstract

Dogs are considered to be man's best friend; they share a close and intense relationship with humans and have shown to excel in reading human social cues, including facial cues. Due to this intimate relationship shared between humans and dogs, we try to understand the process behind how dogs perceive human emotions, and see how similar or different this process is when compared to humans. In this study, the neural correlates corresponding to emotional valence were studied using functional magnetic resonance imaging. Several dogs were presented with image and video stimuli of positive (happy), neutral, and negative (angry) emotions while being scanned. Brain responses to the stimuli were scanned and then modulated by the valence ratings of the stimuli (images and videos). Our findings reveal neural patterns of emotion valence processing in the caudate, amygdala, and hippocampus regions of the dogs' brain.

Acknowledgments

This thesis was possible thanks to my advisor, Dr. Gopikrishna Deshpande. I would like to thank Dr. Thomas Denney and Dr. Jeffrey Katz for their time and guidance. To my family, thank you for everything, this is all you!

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List of Abbreviations

AVI Audio Video Interleave

C-BARQ Canine Behavioral Assessment and Research Questionnaire

DARPA Defense Advanced Research Projects Agency

DSLR digital single-lens reflex

FFA Fusiform Face Area

fMRI functional magnetic resonance imaging

IAPS International Affective Picture System

IRB Institutional Review Board

ISI inter-stimulus interval

ITI inter-trial interval

JPEG Joint Photographic Experts Group

MRI magnetic resonance imaging

SPM Statistical Parametric Mapping

T Tesla

TR Repetition Time

Chapter 1
Introduction

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a non-invasive medical imaging technique which requires strong magnetic fields, electric field gradients, and radio waves to picture the anatomy and physiological processes of the body [1]. MRI does not use damaging radiation and X-rays, and thus has been widely used in the field of radiology, disease diagnostics, and in medical research as well. MRI is based upon Nuclear magnetic resonance (NMR) [2]. NMR is the phenomenon that occurs when electromagnetic radiation is absorbed and re-emitted by nuclei when placed in a magnetic field. Since humans and biological organisms contain hydrogen atoms in abundance, these hydrogen atoms are used to generate a detectable radio-frequency signal in MRI.

An MRI scanner consists of a large superconducting electro magnet that generates a strong and uniform magnetic field which is thousand times stronger than Earth's magnetic field. The magnetic field generated can be varied by the gradient coil such that each location has its own resonance frequency. The radio frequency coil introduces additional energy into the system, in the form of radiofrequency (RF) pulses. At the right resonant frequency, the hydrogen atoms absorb the energy and move to a high-energy state. The affected hydrogen atoms then go back to their original state when the RF pulse is turned off, and in the process releases energy which is detected by the radiofrequency coils in the MR scanner to obtain the raw data matrix. The data is collected in Fourier space, known as K-space in MRI literature [3]. Inverse Fourier transform is used to transform the K-space information into image domain, and spatial information of

the body scanned can be recovered. The change in MRI signal is called relaxation and is classified into T1 and T2 relaxation. The relaxation time varies for different protons in different tissues in the body, thus constructing images of different contrasts. The ability to create contrast between different biological tissues enables MR imaging to generate an image of good quality and high resolution when compared to other medical imaging techniques such as Computed Tomography (CT) and X-rays [4].

Functional MRI

Human brain is the most complex organ of the body. It is capable of processing and storing information, and perform complex functions and yet the inner workings of the brain remains a mystery. However, in recent years, due to the advancement in medical imaging techniques like fMRI and MRI, our understanding of the brain and its functions has significantly improved [5] [6] [7] [8]. fMRI is a non-invasive technique, meaning that the subject isn't exposed to any harmful radiation, or is required to take shots, or ingest substances.

When the body performs a task or an action, the brain responds to that particular stimulus and becomes active. When the brain is responding to a stimulus, the rate of flow of blood to the regions involved in the stimulus grows. This increase in the blood flow helps carry more oxygen to the relevant regions in the brain. The brain uses this oxygen for breaking down glucose molecules which generates energy for it to perform its activities. Blood contains a component named Hemoglobin, which supplies the oxygen to the brain. Hemoglobin is a paramagnetic material which means that it exhibits magnetic properties, when placed in a strong magnetic field. Oxygenated Hemoglobin(HB) and deoxygenated Hemoglobin(dHB) exhibit different magnetic properties, depending upon whether the blood is carrying oxygen or not. Oxygenated hemoglobin exhibits diamagnetic properties, whereas deoxygenated hemoglobin exhibits paramagnetic properties.

These changes in the HB will cause changes in the local magnetic field applied to the body, this will affect the measured MR signal through the Blood Oxygenated Level Dependent (BOLD) [9]. While responding to a task or stimulus, the neurons in the brain activates, which increases the blood flow, which results in bringing more glucose and oxygen (HB) to replace dHB [10]. This results in an increase in the HB quantity, which results in a stronger MR signal due to the diamagnetic properties exhibited by HB. This relation between the blood flow and neuronal activity is known as Hemodynamic Response (HDR). The HDR is usually delayed by about a second or two after the neuron activates. This is followed by a gradual increase in the HDR and peaks at about 5 seconds and then falls back to the baseline in about 10 to 15 seconds after a slow decay. If the neuron is continuously activated by the stimulus, the peak will form a plateau.

Functional MRI tracks the brain functionality in real time, for a given time of repetition (TR), an image is generated in three-dimensional volume by the scanner. A 4D image can be formed by concatenating all the three-dimensional images generated in a scanning session, wherein the fourth dimension is time. The spatial resolution of a fMRI signal is comparatively good, but the temporal resolution, which is of great importance changes with change in BOLD signal. This can however be limited by using the deconvolution technique [11].
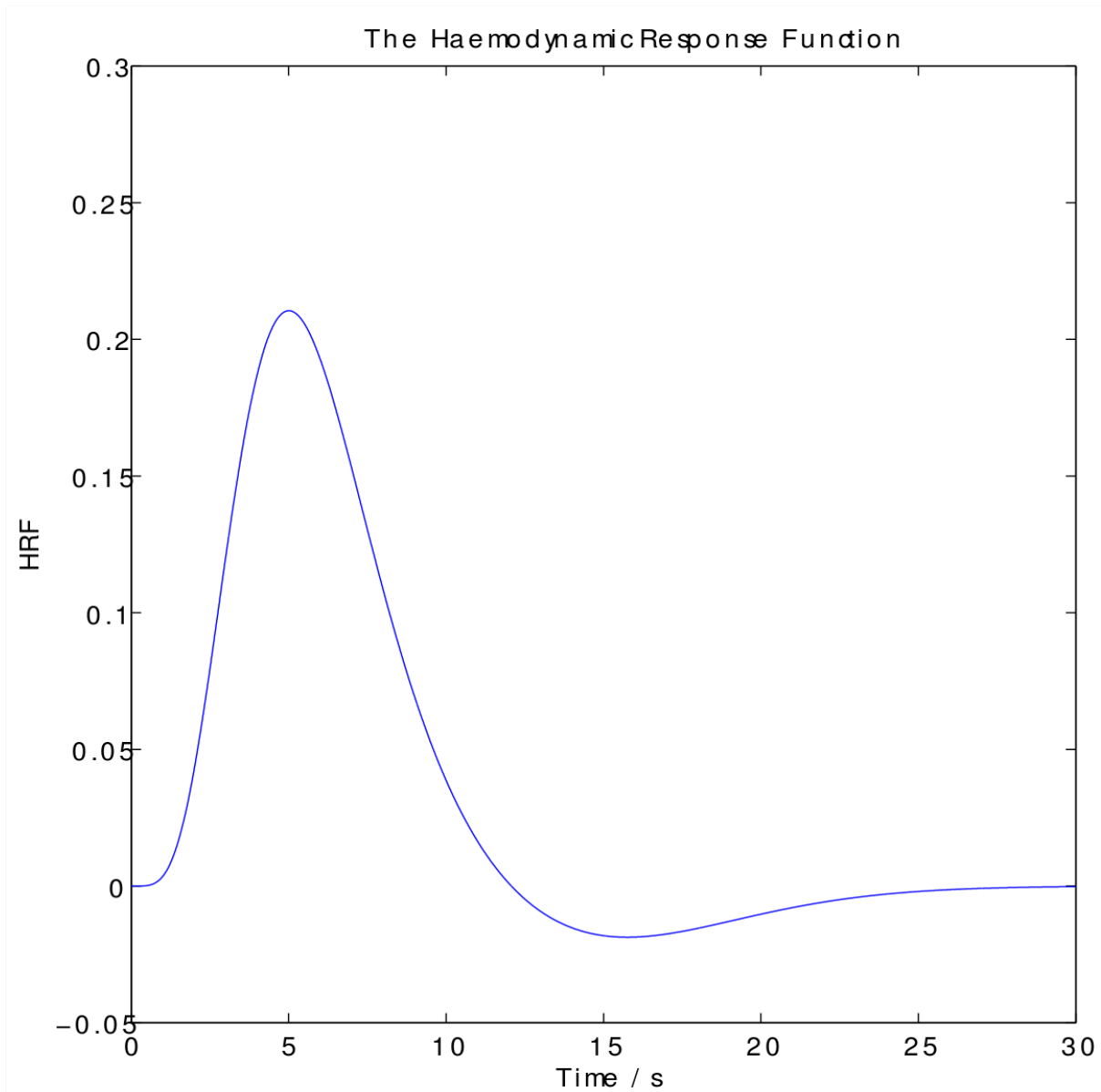
Figure 1. Hemodynamic response function

FMRI Data Preprocessing

　　The raw fMRI data which was acquired from the scanner, contains a lot of noise due to subject movements, random neural activity, and thermal noise and cannot be directly used for functional analysis. Thus, we require standard preprocessing steps to alleviate the noise from the raw fMRI data. In this study,

slice timing, realignment, reslicing, coregistration, temporal filtering, white matter and cerebrospinal fluid removal and spatial smoothing were implemented for preprocessing. The preprocessing steps were performed using DPARSF [12], which is based on SPM [13].

Slice Timing Correction

The output from an MRI scanner is a 3D volume image. This 3D image is captured as series of 2D images over a specified TR. TR is the difference in time between the first image captured and the last image. Since, this time difference is about a few seconds, it is incorrect to assume that all the slices were collected at the same time. This could result in suboptimal statistical analysis, more specifically in event-based experiments. Slice timing tries to correct this by adjusting the voxel time series so that for all the voxels a common reference timing appears. This is done by moving/shifting the time series of values forward or backward by using sinc-interpolation.

Realignment and Reslicing

It is highly unlikely that a subject would stay perfectly skill throughout the scanning process. Even if the subject manages to stay still, the movement caused due to breathing, heartbeat etc. is inevitable. This results in data that is inconsistent in the spatial domain. This also leads to loss of data at the edges of the brain or misleading voxel signals from different types of tissues.

In order to minimize this, the realignment process is implemented, wherein all the images captured are spatially aligned with reference to a single reference image and then rigid body (3 translational and 3 rotational) parameters of each image is calculated by comparing to the reference image. These parameters are used to spatially align all the images.

After the realignment process is done, the registered images are resampled so that all the images captured will match voxel by voxel. This process is known as reslicing.

Coregistration and Normalization

It has been observed that when multiple runs are carried out for a single subject or multiple subjects, the size and shapes of the brain varies with each run or with each individual subject. These differences can result in incorrect analysis. It gets only worse for group level analysis, which considers multiple subjects. Coregistration helps mitigate this problem by spatially aligning the functional and structural images of a subject into anatomical space by mapping the functional information onto it.

Normalization helps coregister different subject's functional images using a standard template atlas for all the subjects.

Temporal Filtering

Temporal filtering helps alleviate physiological noise, thermal noise, magnetic field shifting etc. all these noises tend to fall in a certain frequency band. Since, they are in a certain range of frequencies, these noises can be filtered out using a lowpass or highpass or bandpass filter depending on the frequency range.

White matter and Cerebrospinal Fluid Removal

While measuring the BOLD signal, a common error is observed caused by the white matter and cerebrospinal fluid. This error indicates irregularities in scanner stability, respiration, etc. and may cause overestimation of functional connectivity strength. Corresponding masks can be used to regress out signals from WM and CSF. These masks extract out these errors by averaging WM and CSF signals over all voxels and removing it from the time series.

Spatial Smoothing

      Spatial smoothing is the preprocessing step in which the intensities of nearby voxels are averaged out to generate a smoother spatial map of intensities across the image. The intensities are averaged out by convolving the fMRI signal with a Gaussian function of a particular width. The filter width has to be chosen carefully because, if the filter width matches with the signal width the spatial noise is reduced thereby increasing the SNR. Whereas, if the filter width is large, the spatial resolution of the signal is exacerbated. A FWHM (full width half maximum) of Gaussian kernel of 4mm is most widely used, but if the SNR is still low then a larger FWHM can be used to cover a larger area.

Chapter 2
Social Cognition in Dogs

Canine Social Intelligence

Dogs hold a special place in humans' life as humans and dogs have coexisted with each other for centuries and have a unique social competence, through which they communicate with each other effectively [23]. Many of the facial expression producing muscles in humans are also found in domestic dogs. There is a great amount of data to indicate that dogs excel at reading and understanding human behavioral cues [24] [25] [26].

Unlike non-human primates, dogs have been considered to have unique cognitive skills that are identical to a human infant [27] [28] [29] [30] [31]. The ability to not only discriminate between two humans, but also be able to acquire information from the humans' face from a mere glimpse is extraordinary. Dogs can not only recognize and detect human faces [32], but can also perceive possible outcomes from facial emotions [33]. Studies have shown that dogs can pick up the emotional state from a human face (neutral and smiling faces) [34] and can distinguish those emotional states [35].

Face processing in humans and dogs

Face processing is an important characteristic of social cognitional and human evolution. An individual can retrieve or recall a person's identity and use it for socialization by processing facial information [36]. Across various studies conducted, the activation patterns and neuroanatomical structures of face processing is divided into a core system of processing and an extended system of processing [37]. The invariant traits used to identify an individual activates the

core system, and is activated in the lateral fusiform gyrus, the inferior occipital gyri, and the superior temporal sulcus. The dynamic traits such as eye gaze or emotional expression activates the extended system, and is comprised of limbic regions (emotion processing) and parietal regions (processing spatial information). These systems combined provide highly tuned human expertise in facial information extraction.

Facial cues help provide us with valuable information about emotions or current emotional state and intentions. It is evident from neurophysiological and behavioral studies that a specialized mechanism exists to process emotions of faces [14]. However, processes involving perceiving emotion and discriminating among faces is not the same for all species [15].

In humans, facial expressions in nonverbal communication plays an important role [16] [17]. Humans sensitivity towards other human's facial expressions help them better comprehend the emotions, attitudes, aims, and mood of others. Psychological factors such as personality and empathy affects how humans perceive conspecific facial expressions. Human empathy is generally comprising of cognitive empathy, emotional empathy, and the separation of self from other [18]. Personality tends to affect how humans perceive human facial expressions. Socio-cultural development helps characterize individual human's personality [19]. Personality factors such as extraversion (characterized by persons' activity, assertiveness, positive emotions and enthusiasm) and neuroticism (characterized by self-consciousness, tenseness, moodiness and emotional vulnerability) are often connected to persons' emotional perception. In neuroscientific studies, activation in the amygdala was correlated with extraversion during observation on positive emotional images, and neuroticism modulates the connectivity between amygdala and prefrontal cortex while observing negative emotional images [20] [21] [22].

The first fMRI experiment published to understand face processing in awake dogs [38], the eight fMRI-experienced dogs [39] were presented with movie clips and static images. These dogs had to view the video clips of scenes, human faces, objects, and scrambled objects, for three seconds each. For the static images condition, the dogs had to look at black and white images of dog faces, human faces, scenes, objects, and scrambled faces, for 600 milliseconds each. The fMRI data was processed and investigated for six out of eight dogs and the contrasts for video clips revealed that the inferior temporal cortex in the right hemisphere was involved in human and dog face processing. The data for static images revealed significant category effect when objects and scenes were compared to face images. The scrambled images did not result in a category effect, indicating that the low-level feature processing does not account for activation patterns seen in the temporal lobe.

In another study conducted [40], face processing by dogs was further explored using fMRI. In this study, the dogs were shown 50 images of everyday objects and 50 images of human face displaying neutral expressions. The fMRI data obtained from this experiment revealed two activation clusters for contrast of human faces versus objects. One of the clusters was found to be located in the left temporal cortex and projecting into the frontal cortex, thalamus, and caudate nucleus. The other cluster was localized in the right frontal cortex and projecting into the right temporal cortex. The activation clusters identified in this study were analogous to what has been observed across species.

Investigating Face and Emotion Recognition in Dogs

Although research has been conducted to investigate face processing in domestic dogs, in this study, we try to locate the activated regions in the brain, when faces of varying familiarity and emotional valence were shown to the dogs. Domestic dogs experience visual stimuli in different sharpness and color than

humans [41]. But, considering the close bond that dogs share with humans, do dogs process familiar human faces the same way humans do? Based on previous neuroimaging research with humans and non-human primates, we hypothesized that the dogs would exhibit reliable activations in response to human faces as well as to human facial features related to emotions and familiarity. Based on previous study on non-human primate work, the regions of interest included differential activation in the amygdala which was caused by emotional valence [42].

Hypothesis

In an earlier study conducted by researchers here at Auburn University, wherein the dogs were shown images of familiar and unfamiliar humans with varying emotions (positive, negative, and neutral), and images of familiar and unfamiliar dogs and were simultaneously being scanned. We found that, there were two different regions in dog temporal cortex which were activated. One of those regions when mapped onto human brain [47] [48] was more activated in the Fusiform Face Area (FFA) when the dogs saw human images as opposed to dog images. The other region when mapped onto human brain was more activated in the Superior Temporal Gyrus (STG) when the dogs viewed dog images as opposed to human images.

This difference in the activated regions could be because the dogs did not exhibit any emotions whereas the humans did. The dogs were shown images and videos of humans with positive, negative and neutral emotions in two separate runs. Each of these images and videos have their own individual respective valence scores. In the present study, using these valence scores and the fMRI data acquired, we tested whether the activity in these regions would parametrically modulate with the valence scores. If they were found to modulate with valence, it would mean that these regions cannot be attributed to emotion alone.

Chapter 3
Visual Stimulus Development

Various stimulus sets have been normed for affective quality among humans. These sets could be developed specifically for certain groups such as military [43]. Normed stimulus sets are developed based on ratings from a huge group of scorers on one or more dimensions. The International Affective Picture System (IAPS) used valence, dominance, and arousal as three dimensions of assessment. Scores for arousal were obtained using a continuous scale, wherein the scorers could select an appropriate condition on the scale, ranging from a sleepy figure to an excited figure. The valence dimension was scored ranging from frowning to smiling and the dominance dimension was scored ranging from large self to small self.

A standardized stimulus set was developed for this particular experiment [46].Scored stimuli was used to ensure that the dogs were shown faces varying in emotional valence (negative to positive). Human faces that varied in familiarity and the emotional valence dimension were used as stimulus set in this study and these values could then be used in brain analyses. For familiar stimuli, faces of the dog trainers were used and for unfamiliar stimuli, faces of strangers were used. Irrespective of familiarity, all humans expressed positive (happy), neutral and negative (angry) emotions for both still images and videos.

Stimulus Acquisition

Still Images: This stimulus consisted of both familiar (trainers) and unfamiliar faces of humans. Each individual demonstrated neutral, positive (happy), and negative (angry) expressions, regardless of familiarity. Maximum possible emotion was captured from each individual. A Canon Rebel XT 8-

megapixel DSLR camera was used to acquire the images. The images were then cropped to 600 x 600 pixels framed around the face and neck and were stored as JPEG files.

Videos: Similar to the images condition, for videos we used both familiar and unfamiliar humans expressing positive, neutral, and negative emotions. For the positive emotion, the individuals said, "Good dog!" repeatedly in a tone of excitement and expressing a lot of happiness. For the neutral condition, the individuals were told to repeat, "we're gonna do this. We're are gonna do that.", this was done to avoid any use of potential 'trigger words'. This was done in a monotone voice and no emotion was expressed. For the negative condition, individuals repeatedly said, "bad dog!" with a harsh voice and with anger. A GoPro Hero 3 camera was used to capture the videos and were processed and edited in Quicktime for Mac. The videos were then resized to 1024 x 768 pixels framed around the face and neck and the videos were stored as AVI files.

Scoring: To ensure that the images and videos that were shown to dogs were of the intended emotional valence, raters were used to score each stimulus. They had to identify the emotion displayed and choose the rating of the emotion on a scale from "Very Low" to "Very High". Mean of the scores was then calculated for each individual model's images, thereby resulting in a composite scoring range between -5 (angriest) and +5 (happiest) for each stimulus. The initial images stimulus set was developed by matching the closest possible valence score with the familiar handlers' images to the unfamiliar image. The initial video stimulus set was developed by pairing each of the familiar handlers' videos with the respective unfamiliar video that had the most extreme score (i.e. closest to +5 for positive, 0 for neutral, and -5 for negative).
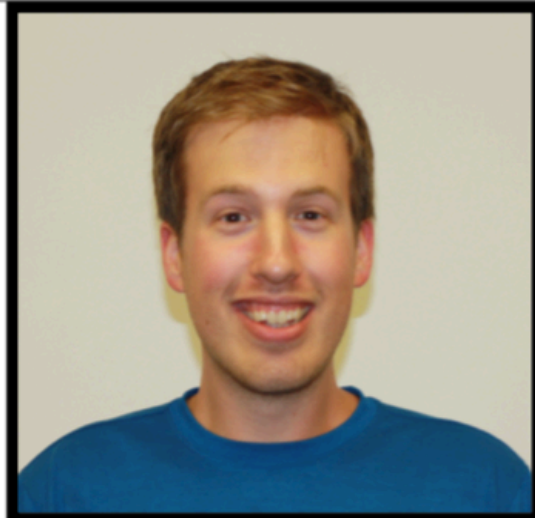
Figure 2. One of the images shown and options to choose from, to score the emotional valence.

Stimulus sets

The initial still image stimulus set varied with each dog group (as familiarity varied with trainers), but each stimulus set had 24 images. Each set consisted of four familiar positive images, four positive unfamiliar images, four negative familiar images, four negative unfamiliar images, four familiar neutral images, and four neutral unfamiliar images. The videos too followed a similar patter, wherein the video stimulus set varied with each dog group (according to trainer familiarity), and each set was made of 24 videos. In each set, there were four familiar positive

videos, four positive unfamiliar videos, four negative familiar videos, four negative unfamiliar videos, four familiar neutral videos, and four neutral unfamiliar videos.
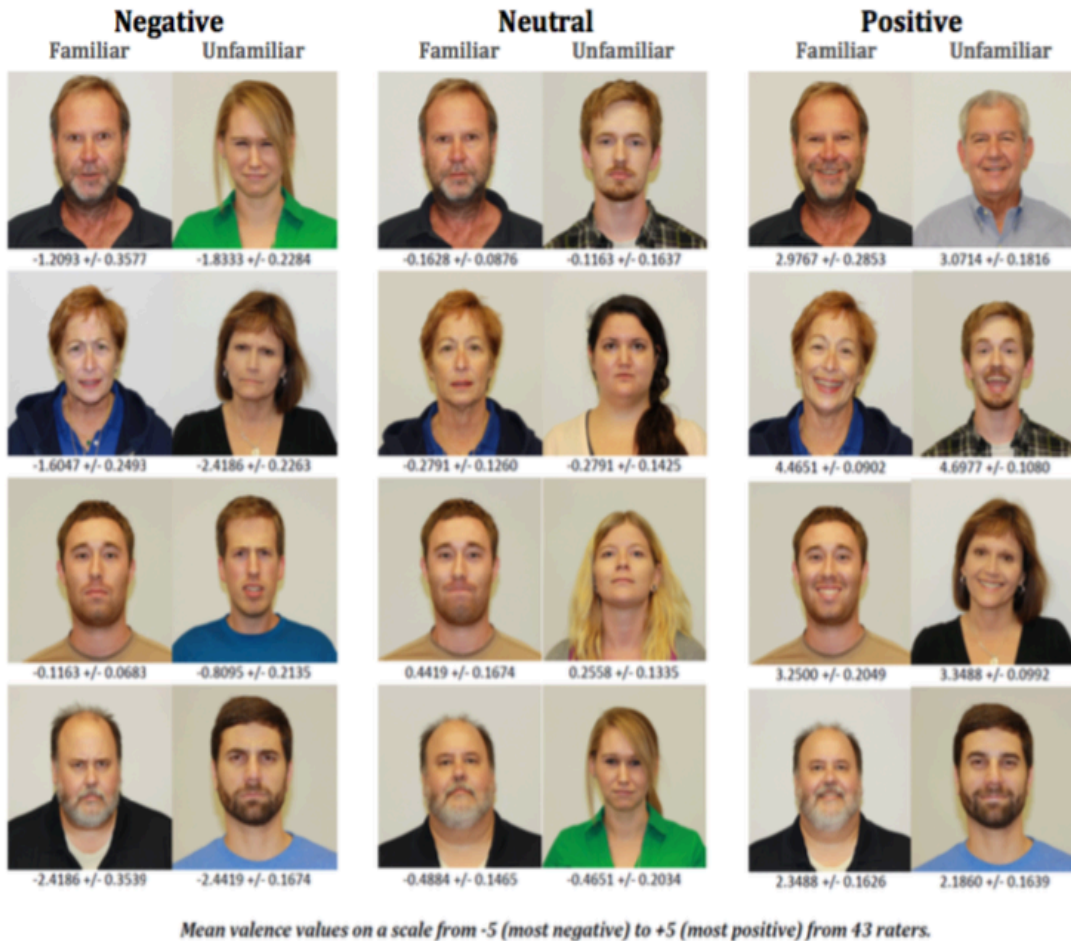

Figure 3. An example of still images stimulus set. Valence scores of emotions were used to match familiar and unfamiliar images. Eight positive, eight negative, and eight neutral images were used in each stimulus set.
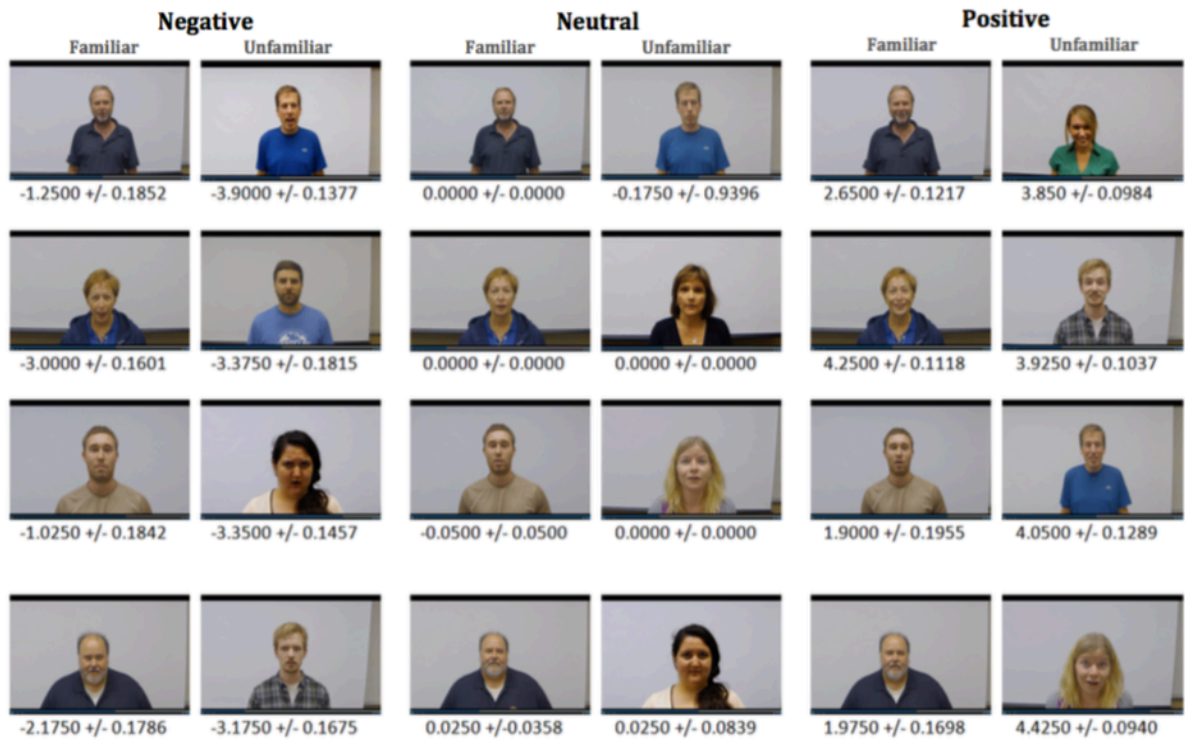
	Negative		Neutral		Positive	
	Familiar	Unfamiliar	Familiar	Unfamiliar	Familiar	Unfamiliar
	-1.2500 +/- 0.1852	-3.9000 +/- 0.1377	0.0000 +/- 0.0000	-0.1750 +/- 0.9396	2.6500 +/- 0.1217	3.850 +/- 0.0984
	-3.0000 +/- 0.1601	-3.3750 +/- 0.1815	0.0000 +/- 0.0000	0.0000 +/- 0.0000	4.2500 +/- 0.1118	3.9250 +/- 0.1037
	-1.0250 +/- 0.1842	-3.3500 +/- 0.1457	-0.0500 +/- 0.0500	0.0000 +/- 0.0000	1.9000 +/- 0.1955	4.0500 +/- 0.1289
	-2.1750 +/- 0.1786	-3.1750 +/- 0.1675	0.0250 +/- 0.0358	0.0250 +/- 0.0839	1.9750 +/- 0.1698	4.4250 +/- 0.0940

Figure 4. An example of the video stimulus set. Unfamiliar humans with the most extreme valence scores were paired with familiar humans' videos stimulus. Eight positive, eight negative, and eight neutral videos were used in each stimulus set.

Expanded Stimulus Sets: Using the methods described above, an expanded stimulus library was developed. This stimulus library included new iK9 personnel. 88 undergraduate students from Auburn University provided ratings online using a Qualtrics survey for which they were rewarded an extra credit by the Department of Psychology Research participation program. The participants had to rate each stimulus based on the emotional valence using the methods described as before. Final valence scores were developed for each stimulus by averaging out (N=88) the responses. The scores can be seen in Table 1.

AUBURN UNIVERSITY

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.

<u>What will be involved if you participate?</u> 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.

<u>Are there any risks or discomforts?</u> 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.

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

<u>Will you receive compensation for participating?</u> 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.

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Figure 5. Form of consent for online participants.

Figure 6. Instructions and questionnaire for online participants.

Table 1

Valence scores for Stimuli rated 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				

Chapter 4

Parametric Modulation of Emotions

In this chapter, we discuss the process, procedures and methods implemented for the visual fMRI task. In this task, the dogs were shown positive, negative, and neutral facial emotions of both familiar and unfamiliar humans. The aim of this study was to check if the neural activity as measured by BOLD fMRI caused in the dog's brain was due to viewing stimuli of various emotions were parametrically related to the valence scores of the stimuli. This was done by modelling a stick function with emotional valence as the parameter. The amplitude of this stick function was the same as the valence scores of the emotions (see figure 7). Though a direct behavioral measure was not provided by this task, we know from previous study that dogs can discriminate between facial emotional content [44]. The differential activation patterns that were caused by emotional valence of stimuli were used to study the neural mechanisms of dogs sensitivity to emotions. *Figure 7.*A mock setup used to train the dogs. This setup helped simulate the scanning environment for the dogs.
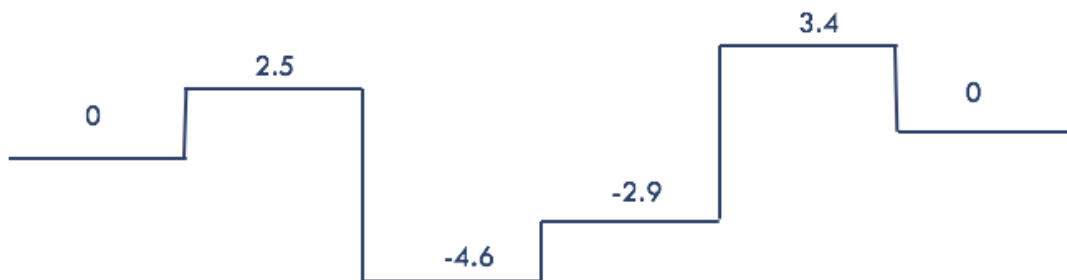

Figure 7. Stick function showing varied amplitude depending on the valence scores of the emotion.

Preparation of Dogs

44 dogs aged between 12 and 60 months were used for this study. All of the dogs were raised in the Auburn Ethical approval for the study was obtained from Auburn University Institutional Animal Care and Use Committee. The methods used in this study were performed as per the guidelines and regulations.
The dogs in this study had to undergo the following training regime.

MRI Training: The dogs were trained to remain still and awake in an MRI scanner . Several positive behavior reinforcement procedures were used to make sure that the dogs remained calm and motionless while being scanned. This also help them stay calm despite the scanner's loud operating noise. The dogs were trained to insert their heads into the human knee coil (in prone position) so that they could be scanned. The training [9] regime for each individual dog lasted about two months and was depended on the particular subject in case.

Clicker/treat method was used for behavioral shaping of the dog. In clicker training, a food reward is paired with a "click" in order to enforce appropriate behavior. The click and treat method was presented at a rapid rate, initially for every 2 seconds as long as appropriate behavior was maintained. This time interval gradually increased till the dog behaved appropriately for several minutes. Appropriate behavior in this study was for the dog to remain motionless in prone position with its head in the coil for 3 to 5 minutes. Once the dog demonstrated good behavior and ease in the scanning room, the dog was deemed ready for functional imaging.

Figure 8. A mock setup used to train the dogs. This setup helped simulate the scanning environment for the dogs.

Figure 9. Further training was conducted for the dogs to transition from the mock wooden setup to an actual MRI scanner. Use of the knee coil can be seen above.

Experimental Design

The dogs were scanned in a 3T Siemens Verio scanner using the human knee coil as a dog head coil. To track head motion in the dogs an external infra-red camera was used, this also help correct for any motion artifacts in the data. Using an EPI sequence, the functional data was generated from the scanner with the following parameters: repetition time (TR) = 1000ms, echo time (TE) = 29ms, 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 had two runs of images and two runs of videos, thus completing four runs in a randomized order. Each run included either 12 stimuli (human faces only) or 20 stimuli (human faces and dog faces) and lasted for a total of 140 seconds. The 12 stimuli included two familiar faces expressing positive emotion, two unfamiliar faces expressing positive emotion, two familiar faces expressing negative emotion, two unfamiliar faces expressing negative emotion, two familiar faces expressing neutral emotion and two unfamiliar faces expressing neutral emotion. For a select few dogs (N =12), still images of dog faces were also shown. The images were presented via projector screen for five seconds followed by a blank screen for a variable 3 to 11 second inter-stimulus interval (ISI). A TR (repetition time) of 1 second was used.

The videos consisted of two familiar faces expressing positive emotion, two unfamiliar faces expressing positive emotion, two familiar faces expressing negative emotion, two unfamiliar faces expressing negative emotion, two familiar faces expressing neutral emotion and two unfamiliar faces expressing neutral emotion. Videos were each 5-seconds long clips of humans stating, "Good dog" repeatedly in a happy tone. For negative videos, the humans repeatedly said "Bad dog" in a forceful tone. For neutral videos, the humans repeatedly said "We're gonna do this, we're gonna do that" in a neutral tone.

Attention Scoring

To make sure that each dog looked at the stimuli while being scanned, several precautions were taken. These precautions were necessary so as to assure that only trails in which the dogs looked at the stimuli were analyzed. Multiple raters judged the attention of the dogs via simultaneous video recording of stimuli

presentation and the dog's eye. For each trail, the rater assigned a rating "yes", if the dog's eye was visibly open and a "no" if the dog's eye was not open enough or closed or that the pupil was not visible. Inter-rater relatability was assessed for each trial, and trails with inter-rater agreement of attentiveness were retained for data analysis.
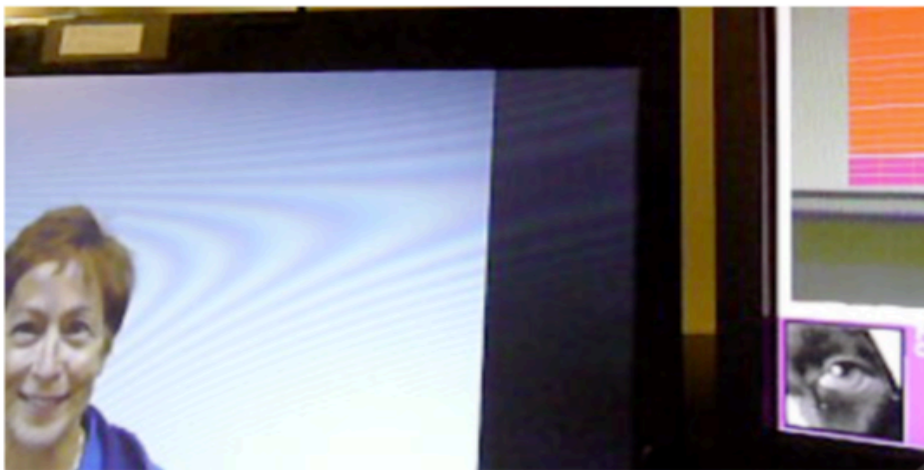

Figure 10. Photo taken of the video which recorded the attention scoring process. The stimulus shown to the dogs can be seen on the left and the video of the dog's eye-tracker can be seen on the right of this image. The stimulus presentation and eye tracking were done simultaneously.

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 ☐

Dog Name: (left margin label)

Figure 11. Scoring sheet used to identify if the dog viewed the stimulus or not.

Data Retention

Of 44 dogs that were drafted for this study, only 30 dogs had usable functional imaging data for both still image and video tasks. Out of these 30 dogs, 7 dogs in still images run and 3 dogs in videos task were not considered due to excessive motion. Data from one dog in still images task and 2 dogs from videos task was deemed insufficient for contrasts. A total of 63 still image runs were obtained for 22 subjects. Of all the images that were shown, more 80% than were rated as seen been by scorers. A total of 73 videos runs were obtained for 25 subjects. Of the videos shown to the dogs, more than 84% of the stimuli were viewed by the dogs.

Table 2

Percentages and counts of images and videos attended to during runs.

Condition	still images		Videos	
	Count	Percentage	Count	Percentage
Positive	204/252	80.90%	246/292	84.24%
Neutral	202/252	80.10%	245/292	83.90%
Negative	199/252	78.96%	247/292	84.58%
total	605/756	80.02%	738/876	84.24%

Image Processing

Pre-processing of the data was done using SPM8. All usable functional data acquired were run the standard preprocessing steps as described in chapter 1. In this study, in order to validate that the neural activity in the FFA correlates to the valence scores of the stimuli, a stick function was modelled. This stick function had an amplitude equal to the valence scores of the stimuli shown to the subjects. Following the preprocessing steps, the general linear model (GLM) was applied and parametrically modulated tests revealed activated voxels for each condition comparison. First level analysis was done for individual subject data. T-tests were performed for positive emotion stimuli against neutral emotion stimuli, and negative emotion stimuli against neutral emotion stimuli. A threshold value of $p < 0.05$ was used. After first level analysis, second level group analysis was performed for positive emotion stimuli against neutral emotion stimuli, and negative emotion stimuli against neutral emotion stimuli. Significant activation

was witnessed in the hippocampus, caudate, and amygdala regions for still image and video stimuli.

Results

Contrasts were devloped between positive versus neutral stimuli and negative versus neutral stimuli for both images and videos. The contrasts from this study were compared to the face processing results [46], wherein we tried to find intersecting or overlapping regions between the two sets of results.

Image Stimuli

We found overlapping regions in caudate for positive versus neutral emotion faces, and overlap in the caudate region and activation in the hippocampus for the negative versus neutral emotion faces.
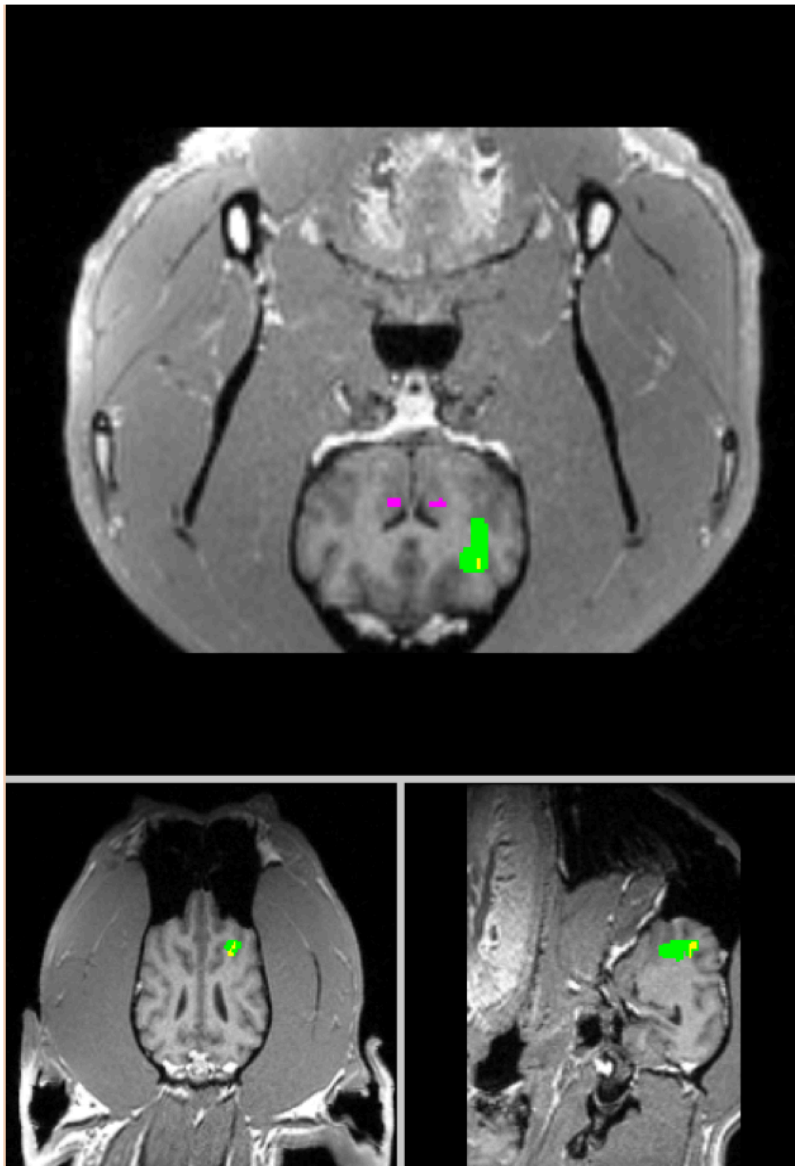
Green – Region of caudate activated by emotion for positive images.
Magenta – Region of caudate activated by parametric modulation of emotion for positive images.
Yellow – Overlap between the two activated regions.

Figure 12. Activation maps for positive versus neutral emotion images. Three orthogonal views are shown for each subfigure.

Green – Region of caudate activated by emotion for negative images.
Magenta – Region of caudate activated by parametric modulation of emotion for negative images.
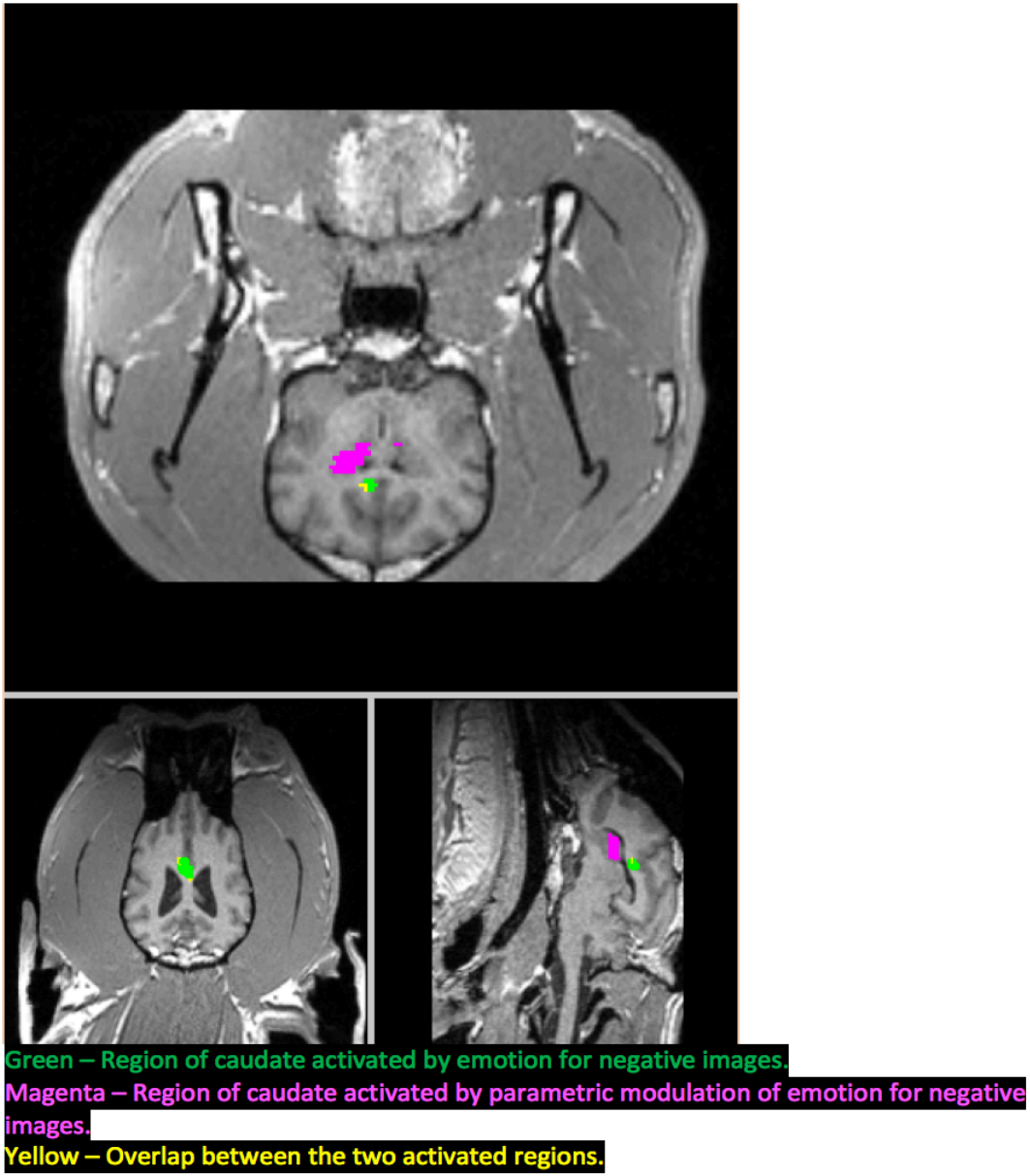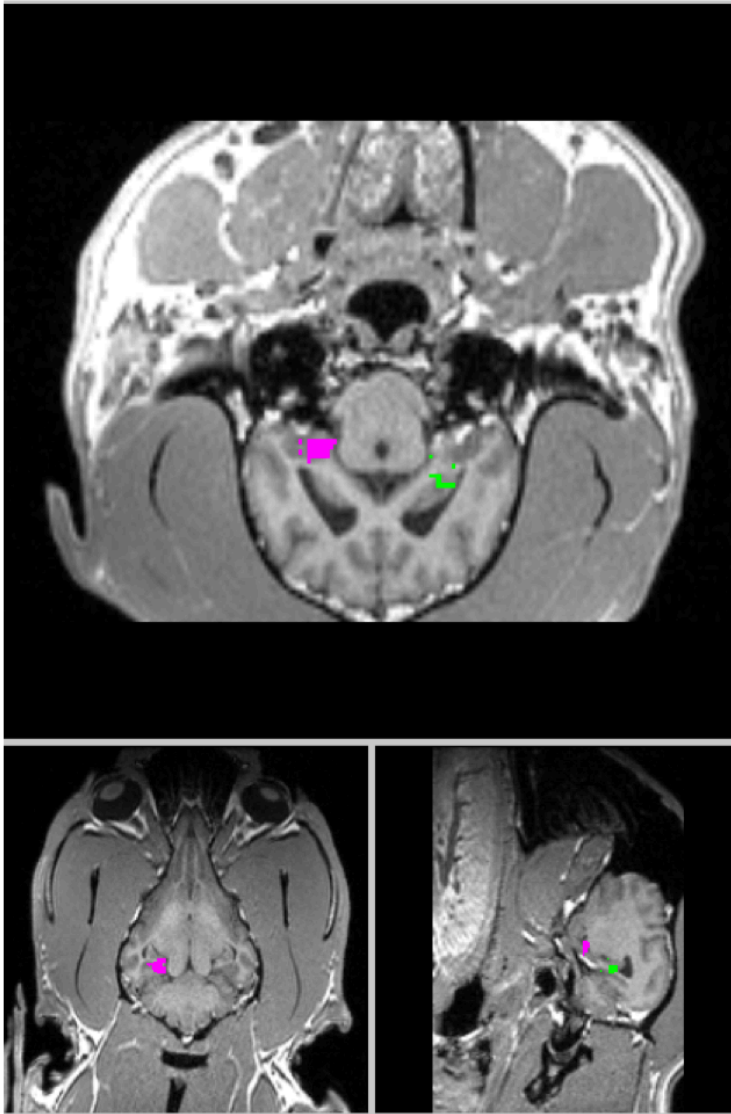Yellow – Overlap between the two activated regions.

Figure 13. Activation maps for negative versus neutral emotion images. Three orthogonal views are shown for each subfigure.

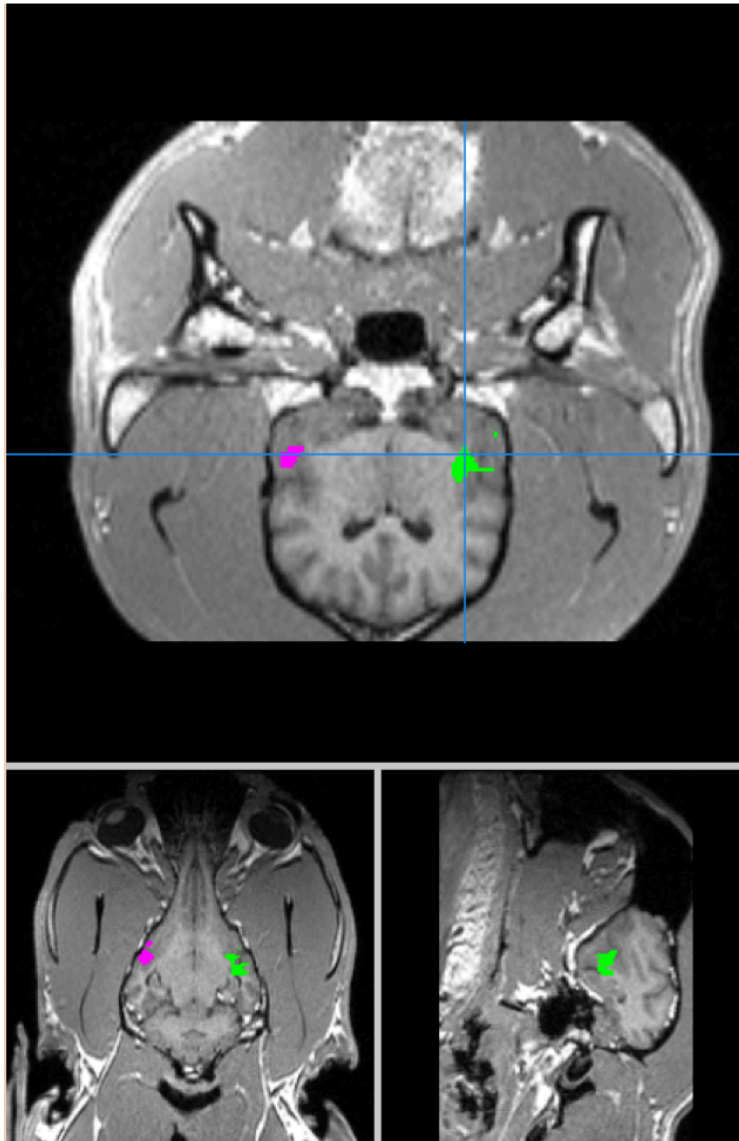Green – Region of Hippocampus activated by emotion for negative images.
Magenta – Region of Hippocampus activated by parametric modulation of emotion for negative images.
No overlap between the two activated regions.

Figure 14. Activation maps for negative versus neutral emotion images. Three orthogonal views are shown for each subfigure.

Video Stimuli

After comparing the results, although we found no overlapping regions, we found significant activation in amygdala and hippocampus for the positive versus neutral emotion faces, and activation in caudate and amygdala region for the negative versus neutral emotion faces.
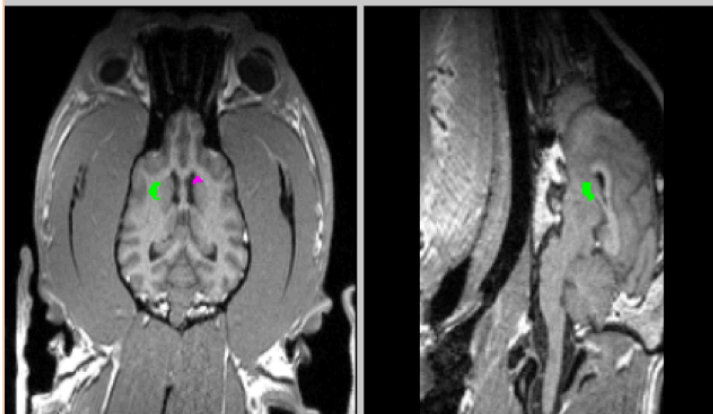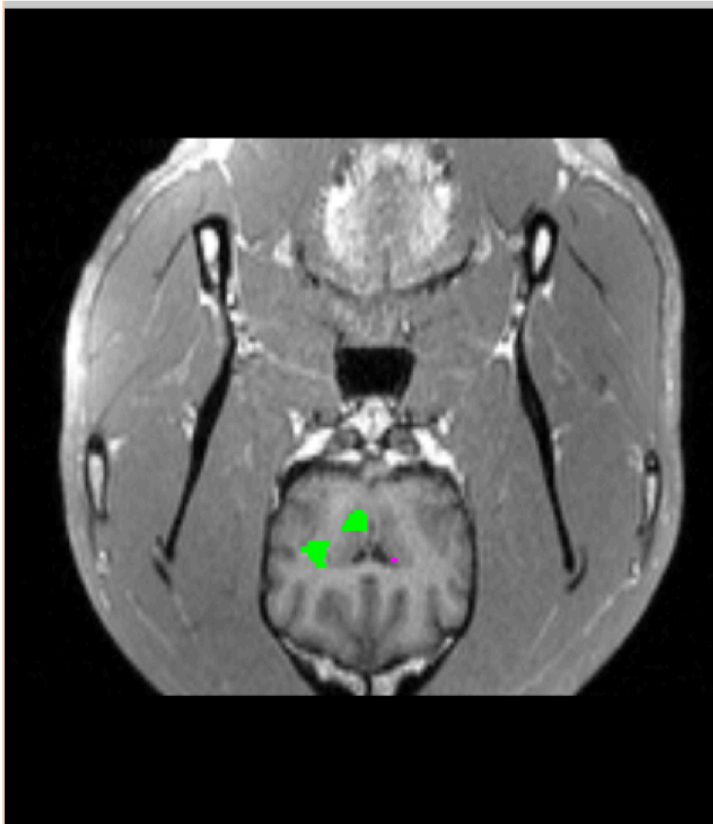
Green – Region of amygdala activated by emotion for positive videos.
Magenta – Region of amygdala activated by parametric modulation of emotion for positive videos.
No overlap between the two activated regions.

Figure 15. Activation maps for positive versus neutral emotion videos. Three orthogonal views are shown for each subfigure.
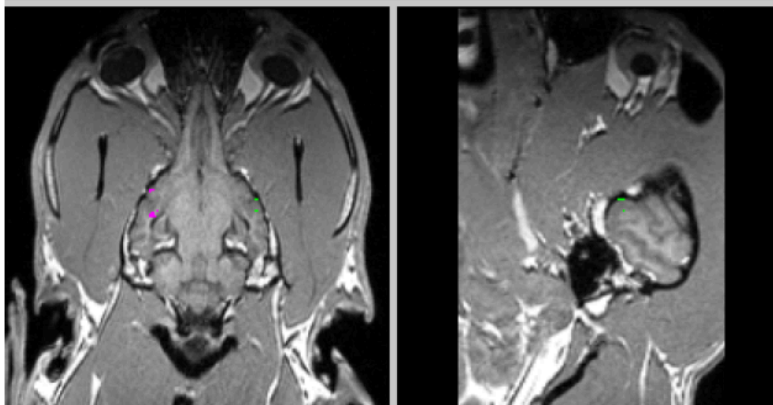
Green – Region of Hippocampus activated by emotion for positive videos.
Magenta – Region of Hippocampus activated by parametric modulation of emotion for positive videos.
No overlap between the two activated regions.

Figure 16. Activation maps for positive versus neutral emotion videos. Three orthogonal views are shown for each subfigure.

Green – Region of caudate activated by emotion for negative videos.
Magenta – Region of caudate activated by parametric modulation of emotion for negative videos.
No overlap between the two activated regions.

Figure 17. Activation maps for negative versus neutral emotion videos.

Green – Region of amygdala activated by emotion for negative videos.
Magenta – Region of amygdala activated by parametric modulation of emotion for negative videos.
No overlap between the two activated regions.

Figure 18. Activation maps for negative versus neutral emotion videos. Three orthogonal views are shown for each subfigure.
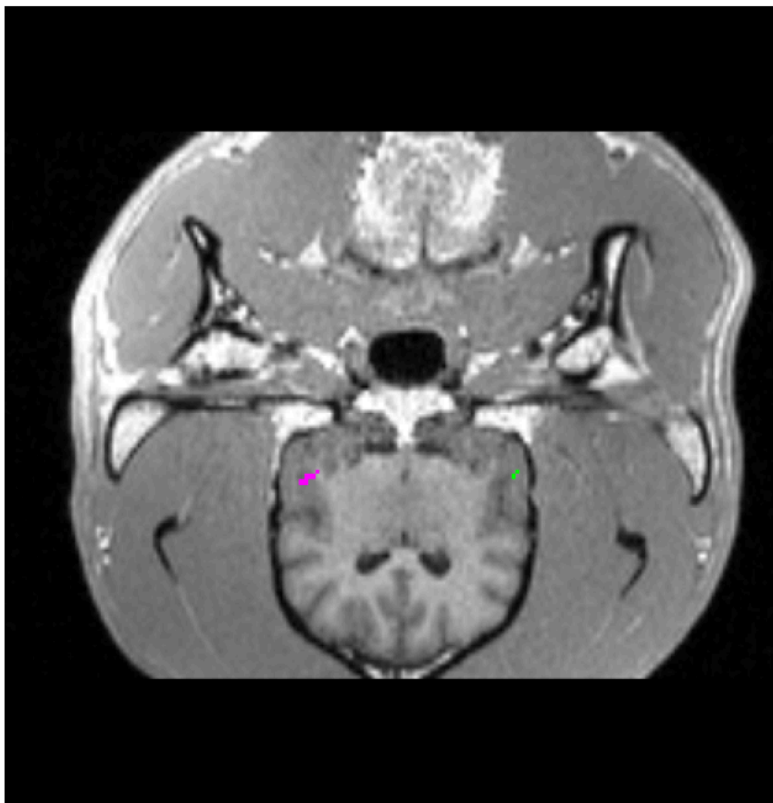
Human Faces versus Dog Faces

To study and understand the effects of intraspecific and interspecific face recognition, contrasts were developed from a subset of dogs which were shown both human faces and dog faces images as stimuli and compared with the results obtained from parametric modulation of emotion.
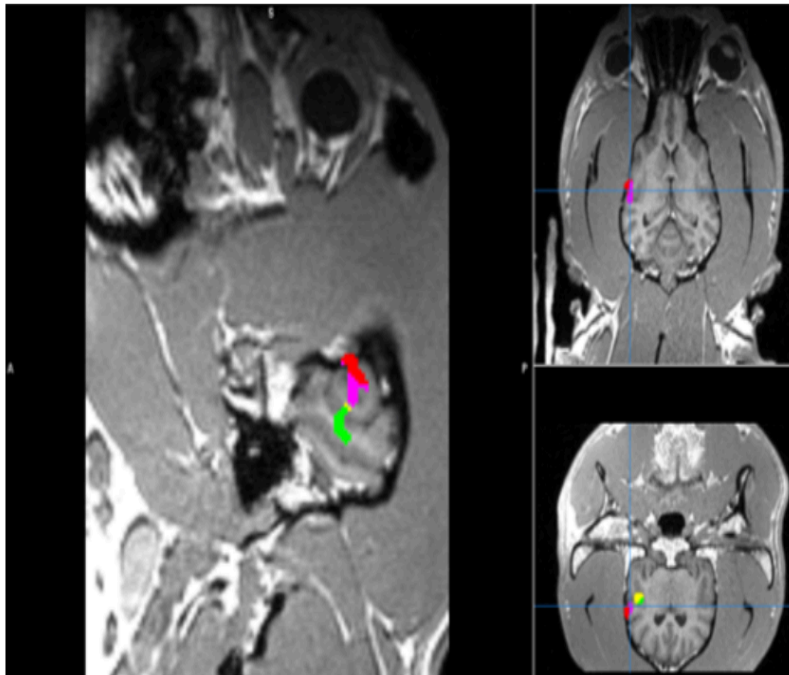
Figure 19. Activation maps showing intersections between HFA and DFA with results from parametric modulation.

Discussion

This experiment was conducted to further understand how canines perceived human emotions and facial expressions with impetus on valence of the emotions. A visual stimulus was developed specifically for the dog sample used in this study. An in-scanner presentation was implemented to image the dogs' neural activity while they watched images of unfamiliar and familiar faces expressing negative, positive, and neutral expressions of varying valence, as well as videos of familiar and unfamiliar humans expressing positive, negative and neutral emotions of varying valence.

This task based experiment was conducted on 40 dogs that were trained to lie still while being scanned. The dogs were shown the both image and video stimuli in two separate runs, varying along emotional valence and familiarity. The data acquired from imaging was then used for the analysis. To see if the regions parametrically modulated with emotions, a stick function was modelled with the emotional valence as the parameter. The amplitude of the stick function was the same as the valence scores of the stimuli viewed by the dog. The post analysis results showed us that regions in the left temporal cortex modulated parametrically with the emotional valence. Neural activations were also observed in the Caudate and Hippocampus for negative emotion images, and activations in the Caudate for positive emotions images were observed. For videos presentations, the neural activations were observed in the Amygdala and Hippocampus regions for positive emotion videos. Whereas for negative emotion videos, the activations were observed in Caudate and Amygdala regions of the brain.

The results obtained in this study lie along the lines of previous face processing research [38] [40]. Previous research of familiar face processing implied the involvement of amygdala, hippocampus, and fusiform gyrus. For

emotion processing, the involvement of amygdala has been implicated. The activations in amygdala and hippocampus in our research may be stronger because of the stimulus emphasis on emotion and familiarity.

Table 3

Comprehensive summary of activated regions and their respective number of voxels

Positive Image Stimuli		
	Activated Regions	Number of Voxels
Positive emotion processing	body of caudate nucleus	796
Parametric modulation of positive Emotions	tail of caudate nucleus	110
intersection or overlap among regions	body of caudate nucleus	268
Negative Image Stimuli		
negative emotion processing	head of caudate nucleus	276
	CA3 of hippocampus	80
Parametric modulation of negative emotions	head of caudate nucleus	300
	CA1 of hippocampus	400
intersection or overlap among regions	head of caudate nucleus	16
Positive Video Stimuli		
	Activated regions	Number of Voxels
Positive emotion processing	CA3 of hippocampus	198
	right amygdala	631
parametric modulation of positive emotions	CA1 of hippocampus	229
	left amygdala	140
intersection or overlap among regions	none	none
Negative Video Stimuli		
negative emotion processing	right amygdala	8
	body of caudate	113
parametric modulation of negative emotions	left amygdala	67
	tail of caudate	16
intersection or overlap among regions	none	none

The findings of this experiment were then compared to the results obtained from a similar study conducted by researchers here at Auburn University [46], in which the human face processing by dogs was studied along the lines of familiarity and emotional state. This previous study conducted, additionally included a subset of dogs which were shown images of both humans varying emotions and dogs. By comparing the results from both the studies and mapping those regions onto human brain, it was observed that there was activation in the FFA of human brain when the dogs looked at human faces when compared to dog faces and activation in STG of human brain when the dogs looked at dog faces as compared to human faces. But, there is data in humans that suggests that FFA is involved in processing emotions in faces. Based on this, we hypothesized that the activation in FFA was caused because the humans varied emotions whereas the dogs didn't. But, judging from the results, both FFA and STG have sub-regions which parametrically modulate with emotion in human faces. So, the specificity of these regions to dog and human faces cannot be attributed to emotion alone. These findings give credence to Nancy kanwisher's Expertise Hypothesis regarding FFA and the role of STG in social cognition [45].

Conclusion

Humans and Dogs share a very intimate and special bond, a bond which was developed due to the close interaction between humans and dogs over many centuries. A bond which was formed by understanding the social, physical, and facial cues of the other species. The purpose of this study was to further investigate and better this bond shared between the two species. This study especially focusses

on how dogs perceive humans and human emotions. This was done by studying the neural indices of human-dog social bond.

The neural processing and correlates of human faces and emotions were explored using fMRI. The acquired fMRI data allowed us to correlate findings that helped better understand the neural functioning of dogs. The results supported and aligned with the preconceived hypothesis of the current study. Activations in amygdala and hippocampus was correlated with varying emotional valence. The findings from this study also revealed that both FFA and STG have sub-regions which parametrically modulate with emotional valence.

The field of canine neuroimaging is still in its nascent stages, with fewer than 20 published studies so far. This study will contribute significantly to the foundations of this interest area. Continued research in this area will contribute to the progress of better understanding the foundations of canine social cognition.

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