Olfaction in Canines - fMRI Study in Fully Unrestrained Awake Dogs

by

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Abstract

For decades, various attempts in vivo and in vitro have been made to explore the anatomical and physiological aspects of the olfactory system in canines and the ways to enhance it. Multiple studies have also attempted to understand the effects of odor detection training in canines.

The current Thesis is aimed at further understanding the ways to improve the existing detection capability of the canines. In an attempt to achieve this, we first have shown that Zinc nanoparticles up-regulated directional brain connectivity in parts of the canine olfactory network. This provides a mechanistic explanation for previously reported enhancement in the olfaction capability of the dogs in the presence of zinc nanoparticles. In this study, we obtained fMRI data from awake and unrestrained dogs while they were being exposed to odorants with and without zinc nanoparticles, zinc nanoparticles suspended in water vapor and just the water vapor. We have then obtained the directional connectivity of the paths between the brain regions of olfactory network that were significantly stronger for the condition of Odorant + zinc nanoparticles compared to just odorant, water vapor + zinc nanoparticles, water vapor. From the results, we observe significant strengthening of the paths which indicates that zinc nanoparticles can indeed be a solution to improve the efficiency of canine detection capability in the realtime environments where the odorant concentrations are very low and would have otherwise been undetected. Then we attempt to explore the effectiveness of the odor detection training on the canines and also the possibilities to improvise the training regime if needed, all the while being able to distinguish a good detector dog from the rest. For this we study the longitudinal changes in the neuronal activity and behavior of canines resulted due to the detection training instead of

the cross-sectional results. The behavioral traits and the fMRI data were obtained at three different timepoints of before the detections training(TP1), immediately after(TP2) and few months after the training(TP3) has been completed in the presence of discriminative and non discriminative odors from the dogs. We hypothesized that the neuronal activity and the behavioral scores significantly varied in correlation from TP1 to TP2 and be maintained same from TP2 to TP3. Further we have explored if the neuronal activity in the olfactory network at TP1 could be used to predict if a dog could be a successful detection dog.

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Chapter 1

Introduction

1.1 **Magnetic resonance imaging**

Magnetic resonance imaging (**MRI)** is one of the non invasive medical imaging technique used for imaging the anatomy and the physiological processes of the body in Radiology (1). MRI scanners make use of strong magnetic fields, radio waves, and field gradients to image the body. MRI is based on the concept of Nuclear magnetic resonance (NMR) (2) which can be explained as the absorption and the emission of the radio frequency energy in the presence of a magnetic field. It is a known fact that human body and other living organisms consists of hydrogen atoms in abundance specially in water molecules or fat molecules. Owing to this fact MRI in clinical and research oriented fields, utilizes hydrogen atoms to generate a detectable radio frequency signal.

Structurally MRI scanner consists large electro magnet that provides a strong and uniform magnetic field. Usually the clinical scanners have superconducting magnets for this purpose. There are also the Gradient coils which are used to vary the main magnetic field thus enabling us to encode the position information of the radio signal received. Then a Radio frequency coil is used to transmit a transient radio frequency signal.

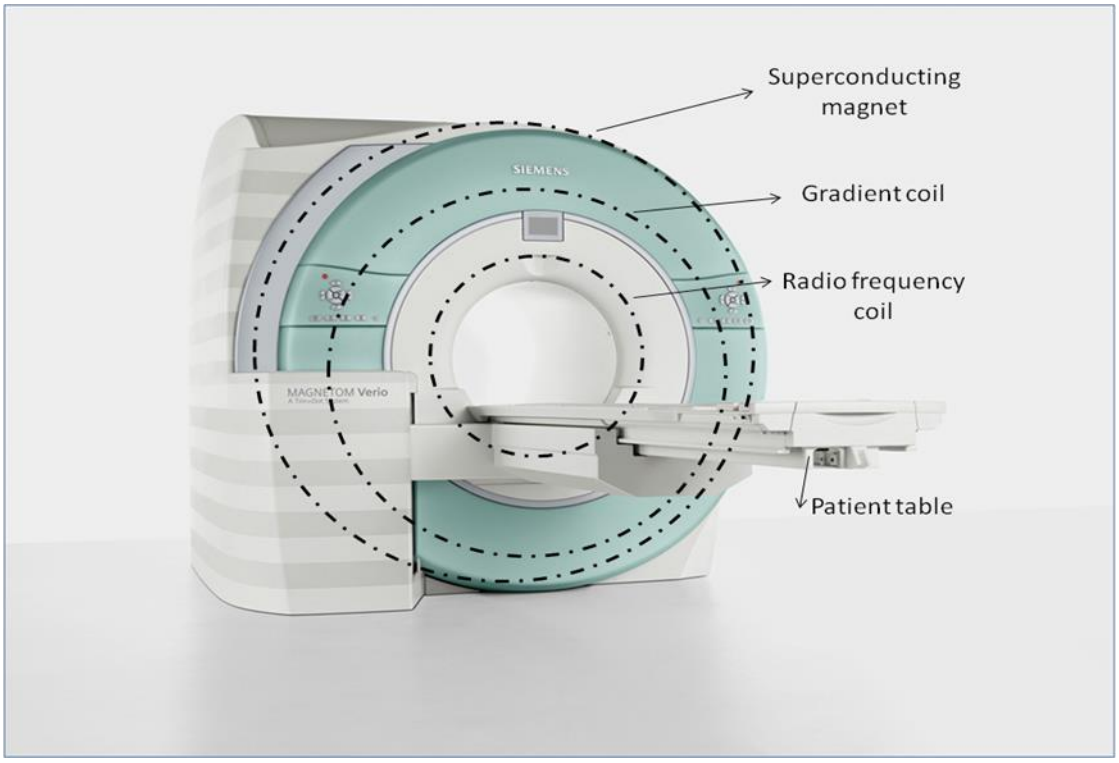

Figure 1.1 Schematic MRI scanner

For scanning a subject is first positioned in the main magnet as shown in Figure 1.1. which results in the alignment of the protons of the hydrogen atoms. Due to the gradient magnetic field protons in each location of the body has a different resonance frequency. Then the RF coil transmits a signal to the body and the energy is absorbed by the protons resulting them to jump from lower energy level to higher energy level hence further resulting in their state of precession in the transverse direction. When the RF pulse is turned off these protons return to their original state realigning to the static magnetic field releasing the energy. The energy that is released by the protons i.e. the MR signal is received by the by the coils to obtain the raw data matrix. From this MR signal spatial information (3) is retrieved using Fourier analysis techniques

and the image of the region of the body scanned is obtained. The MR signal over the time changes referred as relaxations classified as T1 and T2 relaxations explained further in (2). These relaxation times are different for protons in different tissues. In order to distinguish between the tissue of interest from the surrounding tissue we thus use different parameters for scanning purposes creating contrast between the different biological tissues. This property of contrast manipulation enables MR imaging to give a more detailed diagnostic information about the organ compared to other medical imaging techniques like computed tomography (CT) (4) or X-rays.

1.2 Functional MRI

Brain is the most complicated organ of the body. It is capable of so many functions , and how they are done has remained unknown for centuries to us. The invention of imaging techniques like fMRI has finally opened a window to the scientific study of the functionality , organization of the brain (5) (6) (7) (8). One of the main positives why fMRI is dominating this field of research is that it is a non-invasive technique i.e. it does not require the subject to take shots, undergo surgery or to ingest substances or be exposed to any kind of radiations. The FMRI technique measures the neuronal activity in the brain by measuring blood oxygenation level dependent signal (BOLD) in the brain (9).

Brain just like any other organ requires oxygen for breaking down the glucose molecules and thus getting energy for performing its activities. This oxygen to the brain is supplied by the blood component called Hemoglobin. This component exhibits different magnetic properties depending on whether it is carrying oxygen or not. Oxygenated Hemoglobin(HB) behaves like a diamagnetic substance whereas the deoxygenated hemoglobin(dHB) exhibits paramagnetic

properties. The content of dHB changes the susceptibility of the blood which further influences the MR signal. During the brain activity whenever the neuron fires the blood flows in to bring more glucose and oxygen thus replacing dHB with HB (10). To begin with when the dHB is more due to its paramagnetic properties the MR signal is low but when the blood flow increases the diamagnetic properties of HB increase the MR signal. Hence it is implied that the brain /neuronal activity is coupled with the blood flow. This relation is referred as hemodynamic response (HDR). This HDR usually starts about 1 to 2 seconds after the triggering of neuronal activity and peaks at about 5 seconds and then falls below the baseline and finally goes to base line as shown in Figure 1.2. FMRI signal has comparatively good spatial resolution but the temporal resolution is effected by that of the BOLD signal. This imitation can be overcome by using deconvolution technique (11).

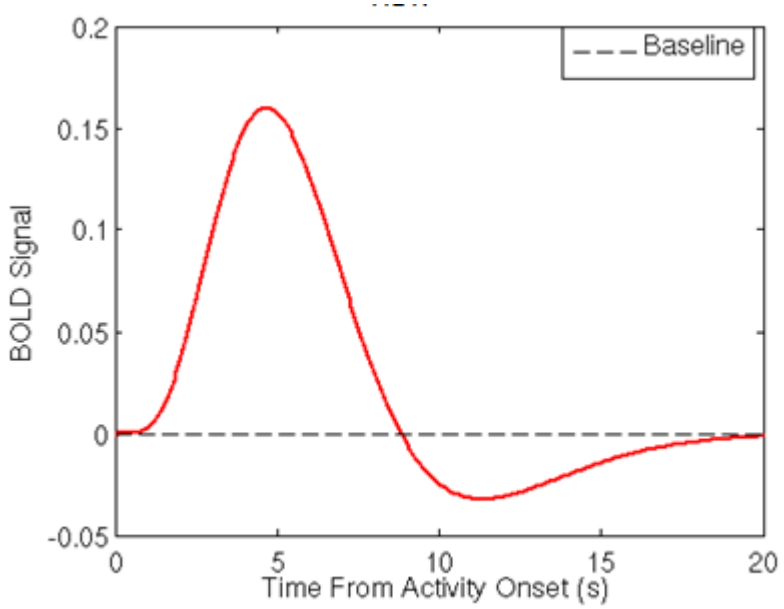

Figure 1.2 Heamo dynamic Response

1.3 Connectivity Analysis

Studies about the brain for the most till nineties of the last century concentrated mainly on the spatial localization and functioning of the brain. It was believed that there were specialized region for each function or tasks or that these functions were associated to corresponding regions. Though this model of approach to study brain has helped a lot understanding simple tasks of cognition , motor etc, it was not effective explaining higher level of functionalities. Hence we needed a more sophisticated approach which considered the information processing network i.e. not just the regions but the networks of the regions and the information flow between them. Basically this model considers spatial localization model, referring to simple functions being processed in specific localized networks and links between these networks contributed to more complex and higher level processes.

The interactions between these regions are believed to be causal i.e. information flow from one region to another where one region causes activity in the other (12) (13) (14) or simultaneous activity in different brain regions which can be caused by the network synchronizations (15). These connections among regions were referred as functional connectivity-FC (simultaneous) and effective connectivity-EC (causal) by Friston (16).

FMRI studies of brain can be mainly classified into two types: Event/Task based and Resting state . The former being the process requiring the subject to perform certain task during scanning and is usually specific to a certain subdivision of the brain. The latter being the signal when the subject is not performing any particular task i.e. baseline activity of the brain. Many studies have functional connectivity analysis done in both Resting state (17) and task based

experiments (18). Effective connectivity was intensively used in task based experiments (19) (20) (21).

1.4 FMRI Data Preprocessing

Raw FMRI data consists of noise due to various sources like thermal noise, system noise, physiological noise, random neural activity and differences in both mental strategies and behavior across people and across tasks within a person. Thus the raw data should undergo the series of standard preprocessing steps typically including slice timing correction, realignment, reslicing, coregistration/normalization, temporal filtering, white matter and cerebrospinal fluid (CSF) signal removal, and spatial smoothing.

1.4.1 Slice Timing Correction

The functional volume of the brain is usually not collected at a single instant but as a series of 2D images. Due to this the time at which each slice is collected at different instance, for example the first and last slices acquired are literally with a time difference equal to TR, or in case of interleaved sequences the time difference even between the adjacent slices is upto half the TR. This difference in time might sometimes lead to suboptimal statistical analysis, especially in event-related designs. In order to nullify this time difference alignment of the slices in the temporal domain is necessary. This slice timing correction can be achieved by simply shifting the phase of the sines that constitute the signal and then resampling the shifted signal using sinc-interpolation. This sub-step of preprocessing lets the slices appear to be acquired at a single instant.

1.4.2 Realignment and Reslicing

Movement of the head of the subject is possible within the run and in between the runs. Even if the subject stay still the physiological noise due to breathing, heart beat etc is inevitable. This motion results in the images being collected in the mismatched locations leading to issues like a given voxel containing signal from two different types of tissue or sometimes loss of data specially at the edges of the brain. In order to avoid this realignment of the images in the spatial domain needs to be done. The realignment of the images is done by setting a reference image(usually the first image or the mean image) and then the rigid body transformation parameters (3 translational and 3 rotational parameters) of each image compared to reference image are calculated. These parameters are then imposed on each corresponding image so that they are all spatially aligned.

Once the realignment is done the resampling of the registered images so that they will be on the same volume of slices and are matched voxel by voxel. This procedure is called reslicing. Any interpolation effected during reslicing is corrected using B-spline method.

1.4.3 Coregistration & Normalization

In order to statistically boost the results of an analysis the data needs to acuired from multiple sessions and sometimes from multiple subjects. Over a group of subjects it is indeed very common too observe the differences in the shape and size of the brains, even with the same subject over the time differences in head profiles are observed for different sessions. However for a group level analysis these differences need to be mitigated or in short normalized to a standard template used as a reference.

Coregistration in general refers to the spatial alignment of functional and structural images from the same subject to map functional information into anatomical space.

Normalization refers to the coregistration of the subjects (mostly anatomical) image to a standard template so as to overcome the issue of brain shape variability from different subjects.

1.4.4 Temporal Filtering

The Noise caused due to the thermal noise, physiological noise, magnetic field shifting etc usually confine to a certain frequency band. So removing the effect of this noise from the required signal can be simply done by using lowpass/bandpass/high pass filters depending on the frequency band. This sub set of preprocessing is called temporal filtering.

1.4.5 White Matter and Cerebrospinal Fluid Signal Removal

At times the gray matter signal might be the only signal of interestand we might need to remove or regress out the signal fromwhite matter and CSF out of the original signal. This is usually done by extracting the white matter and CSF portions using the corresponding masks, taking the mean of the signal from the extracted portion and then regressing it out of the time series of all the voxels.

1.4.6 Spatial Smoothing

Spatial smoothing can be explained as the process of averaging the intensities of nearby voxels to produce a smooth spatial map of intensity across the image. The average of the intensities is achieved by convolving the fMRI signal with a Gaussian kernel of a particular width. This process is done to improve the SNR by suppressing spatial noise, facilitating statistical inference afterwards but it also might reduce the spacial resolution of the signal when the width of the filter is large. Thus the selection of the optimal width for the filter is necessary. It also makes the noise for each voxel more approximately follow the Gaussian distribution. This

is intended since the statistical analysis thereafter is normally based on the assumption that noise follows Gaussian distribution, both temporally and spatially. Grand mean normalization might also be seen employed in preprocessing by a lot of studies. This process normalizes the mean intensity of each fMRI image volume acquired at each TR. This eliminates the disruptive effect caused by grand mean signal intensity bias of each fMRI image volume. This step needs to be carried out before the spatial normalisation.

Sometimes one might encounter field non uniformities, these usually can be avoided by using the shimming coils or by recreating a field map of the main field by acquiring two images with differing echo times. If the field is uniform, the differences between the two images also would be uniform. Bias field can be estimated using a mathematical model of the noise from distortion and used for correction.

1.5 Organization of the thesis

The Chapter 1 of this thesis gives a brief introduction of Magnetic Resonance imaging and Functional Magnetic Resonance imaging followed by a brief description of Connectivity analysis and the basic steps of preprocessing required for FMRI data. The Chapter 2 gives an understanding of the Olfactory system, anatomy i.e. the regions of the brain involved and the paths via which they are connected for the flow of information in canines. The Chapter 3 is about how the zinc nanoparticles can enhance the connectivity of the canine olfactory network when added to the odorants. The chapter 4 explores the longitudinal effects of the odor detection training on the canines. Here we analyze the regions of the brain whose activation could possibly predict if the dog can eventually be a successful detection dog. In the end the Chapter 5 includes the conclusion.

Chapter 2

Background

2.1 Olfactory system

Olfactory system is phylogenetically the oldest evolved sensory system and yet the least understood of all the sensory systems. It is chemosensory system i.e. it reacts with the chemical stimuli(Odorant) in the environment and generates electrical signals which are further transmitted to higher order regions of brain. The processing that occurs in these various regions identifies the odorant and initiates appropriate motor, visceral, and emotional reactions to olfactory stimuli.

2.1.1 Olfactory system Anatomy

The stimuli, called odorants, interact first with olfactory receptor neurons in olfactory epithelium-that lines the interior of the nose. The axons arising from the receptor cells project directly to neurons in the olfactory bulb, which in turn to the projects pyriform cortex in the temporal lobe . The fact that the olfactory system does not take the usual route via thalamus to cortex to process the sensory information makes it unique compared to the remaining sensory systems. Olfactory system also has other forebrain regions like hypothalamus, amygdala involved. The region of thalamus is also involved as the information from pyriform cortex and other regions are relayed to other cerebral and cortical regions through it.

2.2 Olfactory system in canines

The subject of interest in this study are Canines. We need to understand the olfactory path in canines in particular. Based on previous studies involving in vitro and in vivo studies in

canines and other species (mainly humans), we can build the system of olfaction in canines as follows.

The process of Olfaction in canines begins with sniffing, which transports odorant molecules to the nose and delivers them to the mucus layer covering the olfactory epithelium similar to that in humans (22). Here occurs the binding of the odorant by a receptor protein which initiates a cascade of signal transduction events, including the G-protein-dependent production of second messenger molecules, leading to opening of ion channels and passing of ion currents. This process generates an action potential in the axon of the olfactory receptor neuron which is directly projected to the olfactory bulb (OB) (22) (23). The Olfactory bulb mainly acts as a filter but also helps performing the functions of : discriminating the odors , enhancing the sensitivity of the odor detection and lastly filtering out the background odors. The signal from OB is then transmitted to the pyramidal neurons in the olfactory cortex as shown in Figure 2.1. The Olfactory cortex comprises of anterior olfactory cortex, piriform cortex, periamygdaloid cortex and entorhinal cortex. The anterior olfactory cortex detects and has correlates between olfactory features, creating representations (gestalts) for particular odorants and odorant mixtures (24). Pyriform cortex learns these correlations of olfactory gestsalts and a large repertoire of behavioral, cognitive, and contextual information (24). The periamygdaloid area then performs the emotional processing of the information and facilitates memory encoding (25). The entorhinal cortex functions as the hub for the memory network and navigation (24). The signal is then relayed to the regions of hippocampus and thalamus and then to neocortical regions of medial and orbitofrontal cortex for further interpretation (26). According to the previous functional neuroimaging studies in humans the medial and orbital cortex regions are

involved in the cognitive integration of the sensory stimuli (27). The hippocampal region is involved in the recognition of odors from the memory (24). it has been proved that thalamus is involved in odor thresholding, atleast in humans but its fuction of being a relaying hub is still under debate (28). The entire olfactory system of a canine can thus be shown as in Figure 2.1

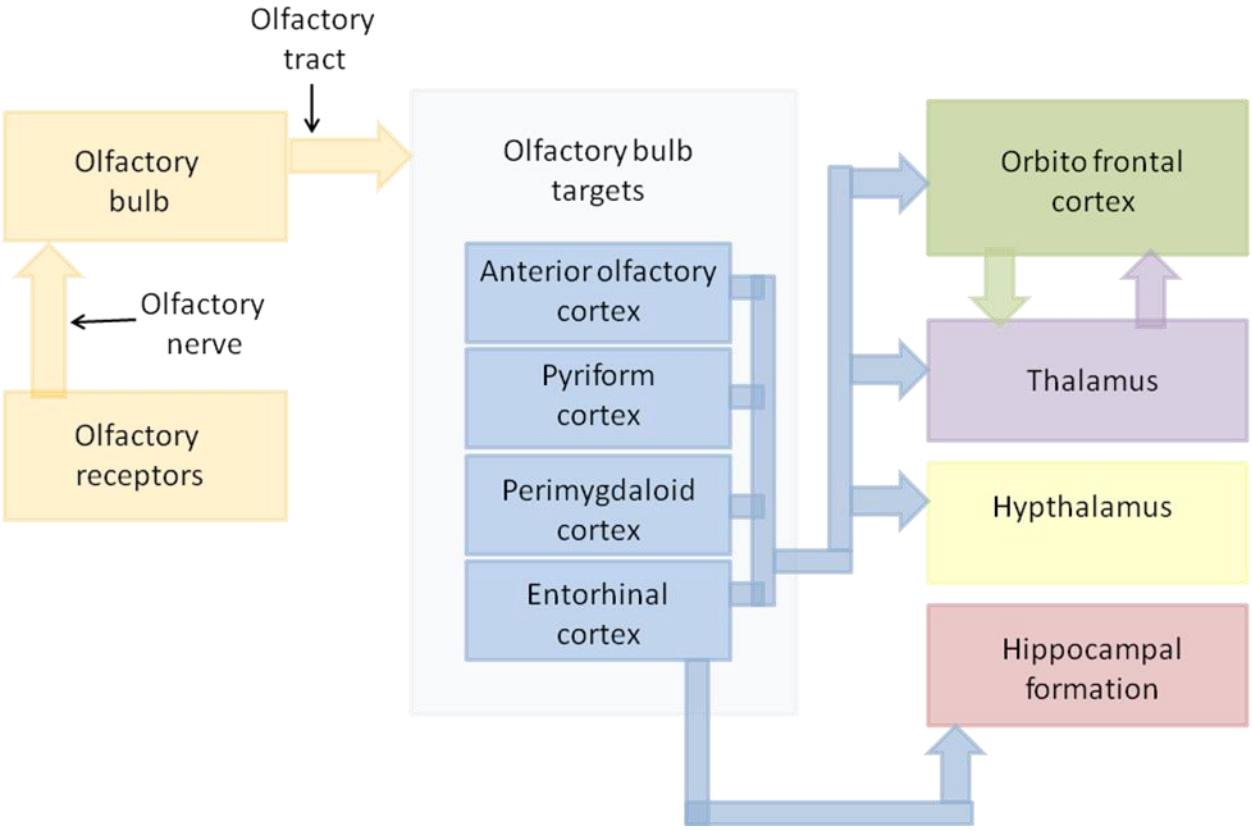

Figure 2.1 Organization of olfactory system in canines

It is noteworthy that previous studies have shown that dogs possess much more olfactory receptors per square centimeter of the olfactory epithelium as compared to humans (29). This could be the reason why dogs have a far more superior olfaction capabilities compared to human beings.

Chapter 3

Zinc Nanoparticles Enhance Brain Connectivity in the Canine Olfactory Network: Evidence from an fMRI Study in Fully Unrestrained Awake Dogs

3.1 Motivation

It is an established fact that canines have superior olfactory capability compared to most known animals including human beings. This makes it obvious why we have been using dogs for detecting different materials in the environment through the history. We have been able to successfully detect and evade dangers in war zones, airports and terrorist targeted public places because dogs have been helping us with detecting explosives (30). Apart from this they have also helped us control drug/narcotics trafficking, tracking people (31) etc. Other detection methods for explosives (32) also exist. These methods have been proven to be effective in controlled lab environments, but when it comes to real environment sniffer dogs still have been the most effective method for this purpose (30). However good sniffer dogs have proven to be an effective solutions there still exist some short comings. One such main hindrance is the concentration of the odorant in the targeted environment. As already discussed olfaction is a chemosensory system where the entire process depends on the chemical reaction of the odorant molecules and the receptor proteins on the epithelium of the nose. The more the concentration of the odorant the higher the number of odorant molecules and the better the accuracy of the detection of the odorant. In some scenarios of extremely low concentrations the odorant might as well be not detected. And in real life situations most of the time the concentrations of the odorants are very low or sometimes be diluted by other over powering scents which makes it

difficult to detect the targeted odorant. Due to this lately there has been new found interest in the research to improve various ways to enhance the odor-related response in dogs. In particular effect of the presence of nanoparticles of various metals such as copper, gold, silver, zinc etc is being actively investigated with not only dogs but other animals as well. Though most of the studies with these metals have shown no particular improvement in the odor detection, it has been noticed that the presence of zinc nanoparticles haave shown significant enhancement in odor responses of olfactory receptor neurons as well as fMRI-based activation in the dog brain (33). These studies have been carried out *in vitro* (34) (35) *in vivo* (33).

Considering the fact that zinc nanoparticles are not toxic at the concentrations used for the dogs (36),it provides a very viable means for enhancing the dogs' odor perception at ultra-low concentrations. Given that the odorants initiate a response cascade in the olfactory network, we hypothesized that a mechanistic explanation for previously reported increased brain activation may be provided by brain connectivity enhancement in the olfactory network of dog brains in the presence of zinc nanoparticles. Below we reconstruct a possible olfactory network in dogs based on previous research which will then form a basis for testing our hypothesis.

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3.2 Method and Materials

3.2.1 Preparation of Dogs

A total number of 8 dogs, aged between 12 to 60 months were used for this experiment. All these dogs were raised in the Auburn University Canine Detection Research Institute. FOr using these dogs for the study, ethical approval was obtained from the Auburn University Institutional Animal Care and Use Committee. All the Institutional and national guidelines for

the care and use of laboratory animals were followed. The current study required exposing the animals to the nanoparticles of the zinc metal, so the concentrations of the zinc nanoparticles the dogs were exposed to were tested to be nontoxic for them (36) making their use ethical in this study.

As we were scanning the brains of a dog in MRI scanner it is required to have the least possible motion in the subjects' head. So these dogs were trained to remain as still as possible with the help of a target stick, in the scanner bed with their heads inserted into the human knee coil (in prone position) for the duration of the scanning, carried out while the dogs were awake and unrestrained. Positive reinforcement behavior shaping procedures were used to keep them as still as possible and to desensitize them to the loud scanner noise. Even before their training in the MRI scanner the dogs were trained to follow, touch and remain touching with their nose the end of the "target stick", a 3/8 inch diameter, 36 inch long, wooden dowel with red tape as target on one end while being given small bits of dog treats as rewards. Accompanying this a tin clinker was used as a condition reinforce to pair the click with delivery of the treat for crrectly holding their nose to the target stick. This training for each dog to reach a proficiency level enough for getting it to train in the fMRI scanner took from 30 to 60 minutes. Before training it in the actual scanner a first round of training was done using a mock scanner using a fixture replicating the human coil (2.5 gal plastic bucket with the bottom cut out) affixed to one end of a table at the height and width of MRI table. once the dog jumped onto the table the tin clicker and target stick routine was used to train the dog to put its head in the mock knee coil. The dogs were trained to hold their heads with least possible movement within the fake coil by clicking the clicker only when their head was in the correct position and held still. The time that a dog had to hold its head

still in the correct position to receive the click followed by a food treat was gradually increased. Throughout this process a recording of the sound from operating scanner was played with gradually increased volumes till the actual scanning volume was achieved. This continued till the subject could hold its head still and in the correct position for a duration of 5 minutes continuously and repeat it multiple times over an hour. Each dog on average took 20 such 1 hour sessions over a 4 week period to complete this phase of training.

The next phase of training was carried out inside the real scanner with the human knee coil while running the actual functional and structural sequences. The dogs were trained to perform the head positioning response in the actual MRI scanner in one, approximately hour-long session each while the trainer accompanied it in the scanning room. The dog was prompted onto the MRI table, to place its head within the human knee coil, and position its nose in the olfactory stimulus delivery mask. Despite the training some head movement is inevitable.

3.2.2 Odorants

The odorant used in the experiment was a mixture of ethyl butyrate, eugenol, and (+) and (−) carvone in water at a concentration of 0.016mM. This odorant mixture, as well as the training procedure, were the same as in (37). The odorant concentration was considered to be 0.016mM as it was the low concentration in the previous work (37),for which the activation of olfaction related areas in the dog's brain could be detected.

The procedure of obtaining and mixing of the zinc nanoparticles is as described in (37). Briefly, zinc nanoparticles were obtained from colloidal zinc suspensions from Purest Colloids, Inc. (http://www.purestcolloids.com/). The suspensions were processed by filtering through 0.22-micrometer Fisherbrand Syringe Filters and centrifuged at 40000 × g for 90min at 18 °C. Atomic

absorption spectra (GTW Analytical Services) was used to survey the concentration of zinc in the suspension. Then, aliquots of the suspension were dried onto coated grids, and dark field transmission electron microscope images (38) (35) were used to count the particles. The suspensions of nanoparticles were then mixed with the odorant and water at a 5nM particle concentration.

The accurate delivery of odor stimulus is very important in any physiological olfactory experiments. A custom made device for the precise computer-controlled delivery of pre-determined quantities of odorants with a precise time interval as described in (37), was used. This device enabled the flow of air under pressure through a series of filters, valves, and manifolds to sweep the headspace over containers into a mask all the while allowing the precise quantitative delivery of odorants to the nasal cavity of dogs. A vacuum suction then cleared the odorant. In this manner, the device controlled the accuracy of extent and time of exposure to the odorants for the subjects. The incoming air flow was limited to 1 l/min based on American Animal Hospital Association (AAHA) guidelines in the mask.

3.2.3 Data acquisition

The data acquisition procedure was described in (37) (33). Briefly, it comprised of the 3T Siemens Verio scanner, the human knee coil adapted as a dog head coil, customized odorant applicator for computer-controlled delivery and evacuation of odorant stimulus, mask for receiving the odorant stimulus and covering the nose and mouth of the dogs, 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.

Data was obtained for each dog while being exposed to the following set of odorants: Odorants+ zinc nanoparticles(OZ), odorants alone (O), water vapor+ zinc nanoparticles(WZ), water vapor alone(W). Every scanning session included 1 run of structural scan, 2 runs of functional scans involving odorant stimulation with zinc nanoparticles, 2 runs with odorant alone, 2 runs of functional scans involving exposure to zinc nanoparticles alone suspended in water vapor and 2 runs of functional scans involving exposure to water vapor alone. These functional scans were run in random order for each dog.

3.2.4 Experimental paradigm

As described in (33), each functional run with odorant stimulus had 5 blocks of odorant exposure each lasting for 10 seconds followed by 30 seconds of rest block to prevent the adaptation of the dog's olfactory response to the odorant. The stimulus block involved the subject being exposed to the odorant that is pumped to the mask. The first 10 seconds of the resting blocks involved vacuuming the odorant from the pipes and the mask followed by 20 seconds of no stimulation. Each run lasted for 200 seconds with the onset times of the stimulant in each run for the 5 blocks being10, 50, 90, 130, and 170 seconds, respectively. The choice of 10-s odor-on condition and 30-s odor-off paradigm was guided by previous studies showing that it is effective for eliciting measurable neural response while preventing habituation.

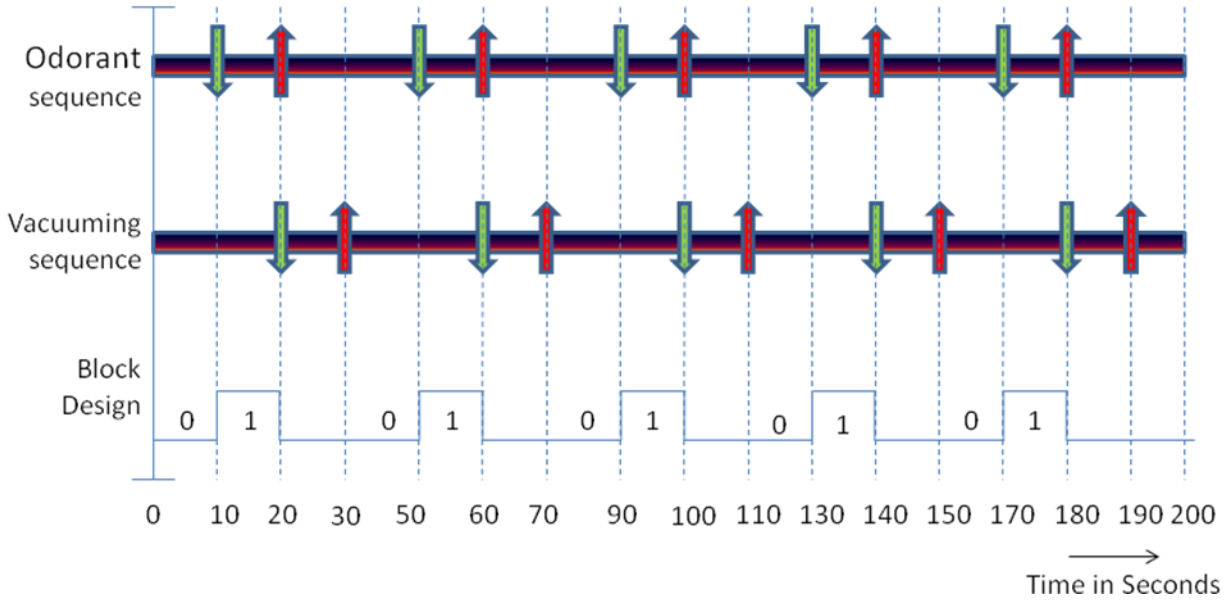

Figure 3.1 *Odorant sequence: Green down arrow indicates the starting of the stimulant presentation and red up arrow indicates ending of the stimulation. ii) Vacuuming sequence: Green down arrow indicates the starting of the vacuuming to remove odorant and red up arrow indicates ending. iii) Block Design: ' '0' indicates the resting state (OFF condition) and '1' indicates the stimulant state(ON condition).*

A schematic of the experimental paradigm is shown in Figure 3.1 and can be explained as follows. In the odorant sequence, green arrows indicate the onset time of the odorant stimulus in the 4 conditions (pure odorants, odorants + zinc nanoparticles, pure water vapor and water vapor + zinc nanoparticles) and down arrows indicate the time when the stimulation ends. In the vacuuming sequence, the green arrows indicate the beginning of the vacuuming or clearance of odorant, and red arrows indicate the ending. The block design represents the paradigm with '0'

indicating absence of stimulus (OFF condition) and '1' denoting the presence of odorant (ON condition).

3.2.5 Data Processing

As described in the (37) preprocessing of fMRI data was done using the software SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/, Functional Imaging Lab, The Welcome Trust Centre for NeuroImaging, in the Institute of Neurology at University College London). The basic steps of slice timing correction, realignment to the first functional image, spatial normalization to a template, and spatial smoothing were done. Then the preprocessed fMRI data was input to a general linear model (GLM) and statistical tests were performed for obtaining voxels in the canine brain which were activated for the comparison of odorants + zinc nanoparticles with zinc nanoparticles alone, water vapor + zinc nanoparticles, water vapor alone were obtained.

Considering the activations obtained from the contrast mentioned above(only the activated voxels) the following Regions of Interest(ROIs) were selected: Amygdala, Hippocampus, Olfactory bulb, Thalamus, Caudate, Pyriform lobe, Frontal cortex. For each of these ROIs, mean time series from activated regions were extracted for every run. These time series were then subjected to blind hemodynamic de-convolution using a cubature Kalman filter and smoother*(Havlicek,et al 2011)*to obtain the underlying latent neural variables. Directional brain connectivity between the ROIs was then obtained for each condition using Dynamic Granger Causality(DGC) by using the analysis framework reported before(*GrantMM, et al, 2015; MD Wheelock, et al, 2014).*Connectivity for all possiblepaths between ROIs for the condition odorant +zinc nanoparticles were computed. Mean connectivity was also computed for each path for the conditions of odorant, water vapor+ zinc nanoparticles and water vapor alone.

Using two sample t-tests, paths whose connectivity strength was stronger for the condition of Odorant + zinc nanoparticles(OZ) as compared to other control conditions of odorant(O), water vapor + zinc nanoparticles(WZ) and only water vapor(W).

3.3 Results

All the paths with corrected $p<0.05$ for the condition of Odorant + Zinc nanoparticles greater than the conditions of Odorant, water vapor + zinc nanoparticles, water vapor (OZ > O, WZ, W) were obtained and are listed in the Table.1along with their connection strengths. The paths are also shown pictorially depicted in Figure.3.2. It can be seen that many paths within the dog olfactory network show strengthening in the presence of zinc nanoparticles.

Table 3.1*Paths with significant increase in connectivity strength for the condition of odorant + zinc nanoparticles (OZ) compared to conditions of odorant (O) , water vapor+ zinc nanoparticles (WZ) and water vapor alone (W).Resultant p-value of the t-test, mean connectivity values of the paths for conditions OZ and (WZ,W,O) are shown.*

Path Origin	Path termination	P-value	Mean Connectivity	
			OZ	O,W,WZ
Amgdala	caudate	8.95×10^{-24}	0.252	0.052
Amgdala	Hippocampus	3.23×10^{-11}	0.194	0.061
Amgdala	Olfactory bulb	1.80×10^{-07}	0.159	0.056
Amgdala	pyriformlobe	3.18×10^{-21}	0.254	0.06
Amgdala	thalamus	5.79×10^{-18}	0.23	0.066
Amgdala	frontal cortex	2.26×10^{-12}	0.217	0.072
caudate	Amgdala	6.16×10^{-20}	0.204	0.053
caudate	Hippocampus	1.74×10^{-28}	0.194	0.013
caudate	Olfactory bulb	2.13×10^{-25}	0.213	0.038
caudate	pyriformlobe	5.13×10^{-18}	0.187	0.038
caudate	thalamus	7.45×10^{-11}	0.156	0.042
caudate	frontal cortex	2.13×10^{-21}	0.218	0.061
Hippocampus	thalamus	4.56×10^{-2}	0.136	0.106
Olfactory bulb	caudate	0.27×10^{-2}	0.162	0.114
Olfactory bulb	pyriformlobe	1.53×10^{-2}	0.154	0.119
Olfactory bulb	frontal cortex	0.04×10^{-2}	0.154	0.099

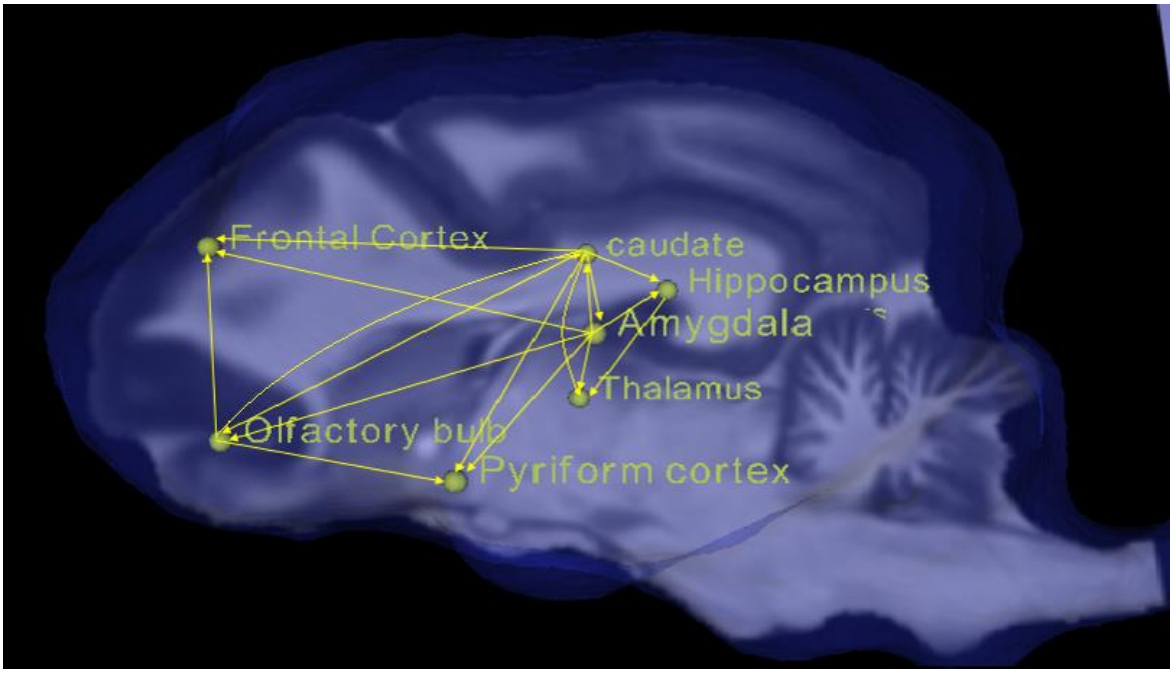

Figure 3.2 *Pictorial depiction of paths with significant increase in connectivity strength for the condition of odorant + zinc nanoparticles (OZ) compared to conditions of odorant (O) , water vapor+ zinc nanoparticles (WZ) and water vapor alone (W).*

3.4 Discussion

Understanding Olfactory system in canines and especially studies of enhancing its functionality have been of high interest in the recent years. Efforts have been made to do the same in vitro cellular (39) (40) or behavioral approaches (41) (42) (43) (44) in vivo (37). In Vivo studies till now mostly concentrated on activations in various regions of the brain.

From these studies Olfaction process can be explained broadly in the sub events of sniffing, chemical binding of the odorant, signal transmission, recognition and interpretation. Each of these events involve different parts of olfactory system (45) (46) (47) in and out of the brain. The olfaction process starts with sniffing which involves olfactory neuroepithelium of the nasal cavity. This enables the transfer of odorant molecules into the nose and to the mucus layer covering the olfactory epithelium (48). This is followed by the chemical binding of the odorant with a receptor protein (49) (50) that triggers an intracellular cascade of signal transduction events of the G-protein-dependent production of second messenger molecules (51) followed by opening of ion channels and passing of ion currents (52). This generates an action potential in the olfactory receptor neurons (ORNs) (53) that is projected to the olfactory bulb (OB) (23). The signal thus generated is then transmitted to the regions of pyriform cortex, periamygdaloid cortex, and entorhinal cortex via olfactory stria. From pyriform cortex and periamygdaloid cortex, the signal is then transmitted to the thalamus and frontal cortex, where it is recognized and interpreted (54) (26). The regions of Hippocampus receive the signal from entorhinal cortex for recognition purposes as well (24) (55). Apart from these, various regions of brain such as the amygdala cortex are involved in the emotional processing resulted from the odors recognized.

As per (33), regions of the brain where activation has been significantly higher in the presence of odorant with added zinc nano particles as opposed to the odorant alone, zinc nano particles alone or water vapor alone were found to be Olfactory bulb, Amygdala, Hippocampus, Thalamus, Caudate, Pyriform lobe, Frontal cortex. So the paths among these regions were analyzed and paths with significant increase in the connectivity strength are obtained.

Olfactory bulb in the olfactory system as explained already receives the signal directly from the receptor neurons and transmits to the amygdala, entorhinal cortex and pyriform cortex. The higher the odorant molecules react with receptor neurons the higher the potential it receives and further trasmits to the above regions. As shown in Table 1 the paths between the OB and Pyriform lobe and to entorhinal cortex have significantly strengthened in the presence of zinc nanoparticles which should be the case of higher concentrations of odorant. Amygdala on the other hand is involved in the emotional processing of the olfactory stimuli((25). As we can see the paths originating and towards Amygdala have enhanced their connectivity in the presence of zinc nanoparticles. Caudate in conjunction with the Amygdala and Hippocampus participates in the funtions related to memory, Goal oriented activities and emotions which are higher order processing of the olfactory stimuli. Thus the increase in the path strengths originating and converging at these parts signify the fact that the presence of zinc nanoparticles in the odorant increases the odor detection capabilities of the dogs. Now coming to the frontal cortex and thalamus, they are involved in the interpretation and recognition

As we can see in the results the paths between almost all the regions involved in the olfaction have significant increase of strength especially the paths originating from Olfactory

Bulb, Amygdala, caudate, Hippocampus when the dogs are exposed to same concentration of odorant but with zinc nanoparticles suspended in it. This represents that the presence of zinc nanoparticles suspended in the odorant has the same effects of having a higher concentrations of the odorant by itself. The paths towards these regions have also shown improved connectivity.

The results of the study thus implicate that zinc nanoparticles enhance the canine olfactory sensitivity thus improving the sniffer dogs detection capability. Also in general this result could have impact on the realms of possible anosmia treatment in human beings.

Chapter 4

Longitudinal assessment of the effectiveness of the odor detection training regime in canines

4.1 Motivation

For centuries, human beings have benefitted from their association with dogs. Dogs have found their use in our lives for various purposes such as herding livestock (66), hunting (67) , guarding, detection and tracking (30) (31) etc.

Current day dogs are most known for their contribution to our society in the field of detection and tracking. We have successfully evaded dangers in warzones, airports etc. thanks to the dogs detecting explosives (30), drugs, people (31), electronics, and biological compounds (68). Over decades, multiple detection devices came into existence with acceptable performance in controlled environments (32), but when it comes to the naturalistic real-time environment, sniffer dogs proved to be still the most effective detectors (30). This is due to the vastly superior olfactory capabilities of dogs over many other organisms.

It is a known fact that the olfactory system has always played a pivotal role in shaping various canine behaviors including their performance as a detection dog and ability to undergo detection training. Many previous studies have been aimed at understanding the canine olfactory system anatomically and physiologically (69) (70) and how they might result in various behavioral attributes in dogs. Recent advancement in noninvasive neuroimaging, specifically fMRI (functional magnetic resonance imaging), have proved to be a powerful tool in

understanding the neural basis of various functions in both humans as well as other animals. Specifically, in the past decade, the feasibility of acquiring fMRI data from awake dogs has been established 37) (71) (72) (73) (74) (75) (76) (77) (78) (79) (80) (81) (82).

Behavioral investigations of dog training and the potential of behavioral instruments for identifying detection dogs have been reported before (44) (83) (84) (85) (86). Previously, attempts have been made to understand the neural basis of olfaction in dogs using cross-sectional experimental designs (37) (33), but these have been correlational in nature. What is missing are longitudinal experimental designs where in it is possible to track neural changes with training as well as predict training outcome using pre-training data. Behavioral instruments have been employed in order to investigate their predictive ability, however, their efficacy has not been clearly established (87) (88) (89) (90). Therefore, longitudinal investigations of neural markers of suitability for detection work are warranted. In this end, a recently published study reported that pre-training activations in the caudate obtained from awake dogs may predict their suitability for service-related assistance work (75). Therefore, we hypothesized that neural markers predicting suitability for detection (as opposed to service) work may be obtained from brain activations related to olfactory processing in awake dogs.

The current study is focused mainly on two objectives. First is understanding and longitudinal tracking of the impact of the detection training regime on neural activity in the canine brain using fMRI. In order to achieve this, fMRI response to odor stimulation in awake dogs was obtained from three time points: Prior to the odor detection training (TP1), after two months of odor detection training (TP2), and six months after the TP2 (TP3). Task fMRI data

was acquired while dogs were exposed to two different odors, Ethyl Butyrate and Eugenol . Canines were trained to detect one of these two odors for a training period of 3 months using a standard training regime. Therefore one of the odors was familiar/discriminative and the other was unfamiliar/non-discriminative to each given dog and discriminative odor assignment to dogs was random. The differential fMRI response to discriminative versus non-discriminative odors was obtained to understand how odor detection training altered neuronal activity in dogs, and whether those acute effects sustained over the long run (6 months).

The second objective is to explore the possibility of using the neuronal activity (specifically, fMRI response to discriminative versus non discriminative odors) in the canine brain obtained before detection training to predict if a canine can become a successful detection dog. In order to achieve this, dogs were divided into two groups based on whether they successfully graduated as detection dogs by the end of the training regime.

4.2 Materials and Methods

4.2.1 Preparation of Dogs

A total of 44 dogs, raised in the Auburn University Canine Detection Research Institute, with ages between 12 and 60 months were used for this experiment. These dogs included both genders and belonged to a variety of breeds like Labrador, German shephers, Alsatian etc Ethical approval for the study was obtained from the Auburn University Institutional Animal Care and Use Committee. We confirm that all methods were performed in accordance with relevant guidelines and regulations.

The canines that were part of this study had to undergo the training regime as described below.

MRI Training: The dogs were trained to remain in the scanner bed with their heads inserted into the human knee coil (in prone position) for the duration of the scanning, carried out while the dogs were awake and unrestrained. Positive reinforcement behavior shaping procedures were used to keep them as still as possible and to desensitize them to the loud scanner noise. This training regime is as described in [9] and depended on each individual subject and lasted for about 2 months in average as shown in Figure 4.2.

Detection Training: All the dogs that participated in this study had to undergo a standard explosive detector dog training regime for preparing fully trained explosive detection dogs. This regime is approximately 3 months long comprising of the initial (pre-training) and operational training phases. It involves training the dogs to discriminate the odors (imprinting) as well as to search the target odors in relevant operational scenarios.

Detection work and maintenance training: The dogs were engaged in maintenance training with operational style search activities at least twice a week. Usually no target odor is present in the activity mimicking actual scenarios of explosives detection work. Occasionally a target odor was presented as a motivation to the dog's vigilance in searching. Maintenance training involving some short and simple odor detection tasks with target odor were carried out to ensure that they will alert to the odors.

At the end of this regime few of the dogs were successfully recruited as the detection dogs and the rest were deemed as unsuccessful.

4.2.2 Behavioral Measures

The dogs were behaviorally characterized based on their ability to hunt, retrieve and be environmentally sound (91)(92). Performance measures for each dog in each of these categories ranged from 1 to 5, corresponding to low and high proficiency, respectively. The final integrated behavioral measure was then obtained as the summed-up scores of these hunting, retrieving, and environmental soundness scores for each dog.

4.2.3 Odorants

In the current experiment, we used two odorants: Ethyl Butyrate and Eugenol at a concentration of 0.016 mM. We used this odorant concentration as it was the lower of the two concentrations of the same odorants used in (37) and was sufficient to cause a detectable activation in olfaction related areas in the dog's brain. This also allowed for familiar odors to elicit a higher response without the response getting saturated. We refer to familiar odors as discriminative odor as the dogs learned to discriminate them, which was not the case with non-discriminative odors. Therefore, discriminative/non-discriminative are better words to characterize the relevance of the odors to dogs than familiar/unfamiliar. The differential activity in the brain to discriminative as opposed to non-discriminative odors might be due to some inherent physical property of the odorant instead of being due to the discrimination of the stimuli. In order to avoid any such issues we trained 22 of the 44 dogs to alert in the presence of Eugenol and the remaining 22 in the presence of Ethyl Butyrate. Thus for the randomly chosen

half the dogs, Eugenol was the discriminative odor and Ethyl Butyrate was the non-discriminative odor and it was the other way round for the rest of the dogs.

4.2.4 Experimental paradigm

The data was obtained for each subject while being exposed to both the discriminative and the non-discriminative odor in different runs. Each scanning session included 2 runs of functional scans involving discriminative odor stimulation and 2 runs involving non-discriminative odor stimulation. All the four scans per session were run in a randomized odor for each dog.

Odorant stimulus was presented using a computer controlled device as described in Jia et al 2014. Every run lasted for 200 seconds with five 10 second blocks where the dog was exposed to the odorant followed by a 30 second rest block to ensure that the dog's olfactory response does not adapt to the odorant. The initial 10 seconds of the rest block was utilized to vacuum the odorants and the latter 20 seconds had no stimulus presented. These 10s stimuli + 30s rest paradigms was chosen as it has been shown to be effective for obtaining measurable neural response [9] [27]. The onset times of each block were 7s, 47s, 87s, 127s and 167s. The schematic for the experimental design is shown in the Figure 4.1.

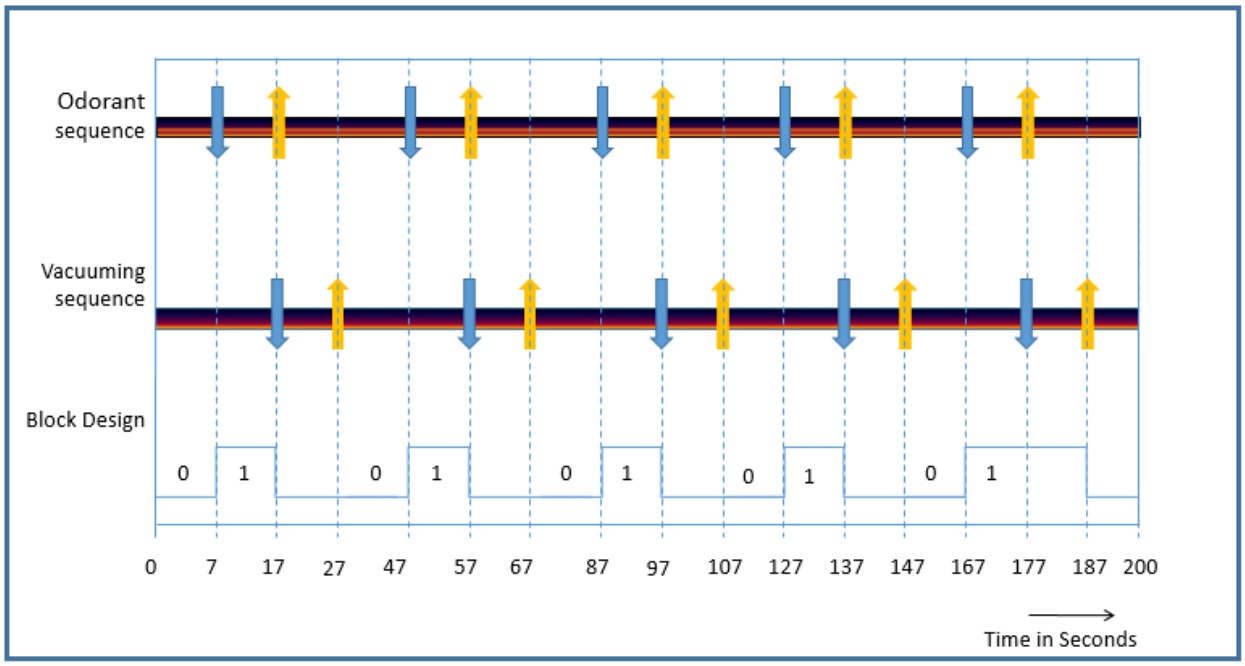

Figure 4.1*: The schematic of the experimental paradigm followed in each run. In the odorant sequence,blue arrows indicate the onset time of the odorant stimulus in both the conditions (Discriminative odor and Non-discriminative odor) and yellow arrows indicate the end of the stimulus. Similarly in the vacuuming sequence, the blue arrows indicate the onset time of the vacuuming or clearance of odorant, and the yellow arrows stand for the end of the vacuuming. The block design indicates the basic paradigm of each run where '0' stands for the absence of stimulus (OFF condition) and '1' stands for the presence of the stimulus (ON condition).*

4.2.5 Longitudinal assessment

In order to identify superior detection dogs which are easily trainable, instead of a cross sectional study, longitudinal assessment of changes in neuronal activity and behavioral attributes as a result of training was performed. The experiment was been designed to take place in 8- 10 months for each dog as depicted in Figure 4.2.

Timepoint 1 (TP1): Prior to the commencement of detection training (but after the dogs were trained to keep their head still in the MRI scanner), fMRI data and behavioral scores were obtained from the dogs in the presence of both the odors, Eugeno and Ethyl Butyrat. These sets of scans/data are considered as Timepoint 1 (TP1).

Timepoint 2 (TP2): After TP1, the dogs were trained using the standard detection training procedure for 3 months. Half of the dogs underwent a training to detect the odor of Eugenol and the rest to detect Ethyl Butyrate. On completion of odor detection training, a set fMRI scans and behavioral scores were obtained and are referred as Timepoint 2 (TP2).

Timepoint 3 (TP3): After TP2, the dogs underwent 4 subsequent months of intermittent detection work and maintenance training. On completion, the final set of fMRI data and behavioral scores were acquired corresponding to TP3.

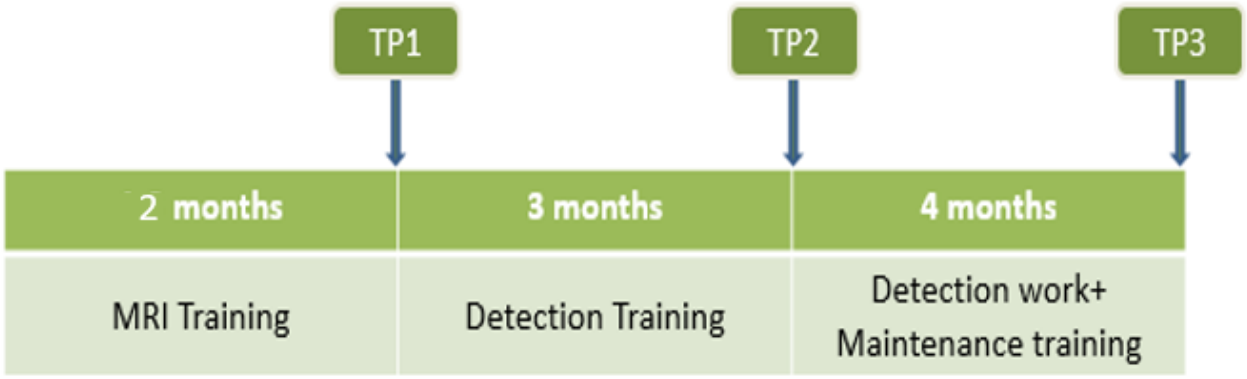

Figure 4.2: *The schematic of the longitudinal experimental design*

4.2.6 Data acquisition

The data acquisition procedure was as described in detail in our previous publications (33) (37). In short, it consisted of the following components: a 3T MAGNETOM Verio scanner (Siemens Healthcare, Erlangen, Germany), a 15 channel human knee coil used as a dog head coil, a computer controlled odorant applicator for the delivery and evacuation of odorant, mask for receiving the odorant stimulus and covering the nose and mouth of the dogs, an external infra-red camera used to track head motion in dogs and retrospectively correct for motion artifacts in the data. Functional MRI data was obtained 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^2, 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.

Among the recruited 44 dogs, four dropped out before scanning commenced. Out of the remaining 40 dogs, we could acquire usable data from 37 dogs in TP1. One of the dogs dropped out after TP1 and from 39 dogs available at TP2 after the detection training, we could get usable data from 37 dogs. Further, 7 dogs dropped out after TP2, resulting in only 32 dogs being scanned in TP3 out of which useful data was successfully acquired from 27.

4.2.7 Data Pre-processing

The fMRI data obtained was first preprocessed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/, Functional Imaging Lab, The Welcome Trust Centre for Neuroimaging, in the Institute of Neurology at University College London). The preprocessing included the basic steps of realignment to the first functional image, spatial normalization, and spatial smoothing. Considering the dog brain being different from the human brain we have adopted few changes.

Normalization: Spatial normalization is very critical in this study due to variations in the sizes of the dog heads. Reporting activations in individual subject space is not an option since such results cannot be replicated or generalized. Standard templates are readily available for humans, monkeys and rodents which are derived from data acquired from hundreds of subjects. Unlike these, the existing anatomical dog brain templates are derived from not more than 10 subjects, which may not capture varying head sizes of dogs. To overcome this bottle neck, we have used the approach as described in (71), where we have selected the best anatomical image available from multiple subjects as a template. A functional image from the same session was then normalized to the template chosen. This transformed image was used as the template for all the functional images from other sessions.

Smoothing: As the dog brain is smaller compared to the human brain, we have used Gaussian smoothing kernel with full width half maximum (FWHM) of 4 x 4 x 4 mm^3 instead of 8 x 8 x 8 mm^3 that is generally used [10].

4.2.8 Data Analysis

4.2.8.1 Activation Analysis and Correlation with Behavior

The pre-processed data was fed to a general linear model (GLM) for regression analysis and statistical tests were done to obtain the voxels active for the desired conditions. The analysis was done using SPM8 as follows. At TP1, a first level analysis for the T-contrast of Odor greater than No odor (O>NO) per subject was performed since all odors were non-discriminative at TP1. For the remaining time points, a first level analysis for the contrast of Discriminative greater than Non-discriminative (D > ND) was performed in every subject.

The group-level statistical activation maps were then obtained using second level group analysis involving the following comparisons using paired t-tests: TP2 and TP1 and TP3 and TP1. This was done to observe changes in activation resulting due to detection training. Here TP2 vs TP1 represents the immediate effect of the detection training program where as the TP3 vs TP1 represents retention of those effects post-training as compared to the pre-training baseline.

The behavior data was obtained from all the three timepoints of TP1, TP2, TP3. The difference of the behavioral score for each dog between TP2 and TP1 were calculated. Similarly, the differences in behavioral scores between TP3 and TP1 were calculated as well. The correlation between longitudinal changes in brain activation and behavior were estimated.

4.2.8.2 Predicting Training Outcome using Pre-training fMRI Data

Depending on whether a given dog successfully graduated to become a detector dog or not, the dogs were divided into two groups: successful and unsuccessful. After quality control checks including screening for excessive motion, a total of 30 dogs had good quality data at TP1 out of which 10 were deemed successful and the rest 20 were unsuccessful at the end of the training regime. The regions of the dog brain where the activation in TP1 for the contrast of Success group> unsuccessful group was observed to be high with no higher activation as the after effects of the detection training (TP2-TP1 and TP3-TP1)were determined. The beta weights of these regions for the dogs at TP1 were determined and used as inputs for predicting the training outcome of dogs. The regions which had higher activation because of detection training i.e. at TP2> TP1 and TP3> TP1 were obtained. The beta weights at TP1 from the above regions were also used as classifiers to see if they can be reliable predictors.

We have used logistic regression as a training kernel to calculate classification accuracy i.e. to see the accuracy with which it is possible to predict if a dog can be a successful detection dog. To validate the classification model on unseen data, 4-fold cross validation was used. In order to evaluate the performance of these models, the receiver operating characteristics (ROC), a plot of true positive rate (TPR) versus false positive rate (FPR) was plotted and the Area under the curves (AUC) were compared for various input features.

4.3 Results

4.3.1 Activation

The T-maps for the contrast of discriminative odor greater than non discriminative odor for TP2 > TP1 i.e the regions that showed higher activation in TP2 compared to TP1 but showed no significant difference from TP2 to TP3 are as shown in the Figure 4.3. The regions satisfying this criterion are the regions of Hippocampus, Amygdala and caudate. Similarly we have obtained the T-maps for the contrast of discriminative odor greater the non discriminative odor for TP3> TP1 but no significant increase from TP2 to TP3 as shown in the figure 4. The higher activation in these regions signify the lasting effects of the detection training on the canines.

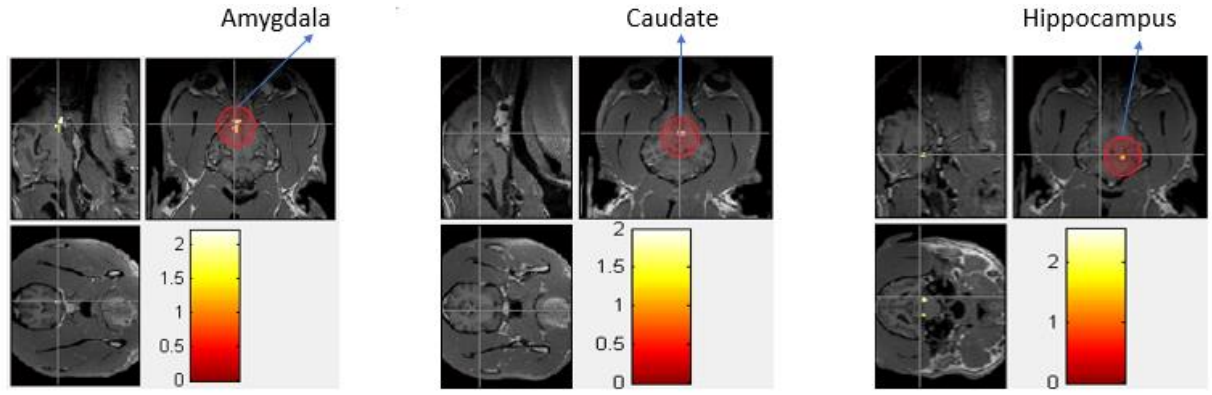

Figure 4.3*: The regions with higher activation for Discriminative odor than the non-discriminative odor in TP2 as compared to TP1.*

Table 4.1: Statistics for the regions active in TP2> TP1 for discriminative odor greater than non-discriminative odor.

S NO	Regions activated	Number of active voxels(41)	Peak T value
1	Amygdala	19	2.1958

2	Caudate	16	2.5579
3	Hippocampus	6	1.9873

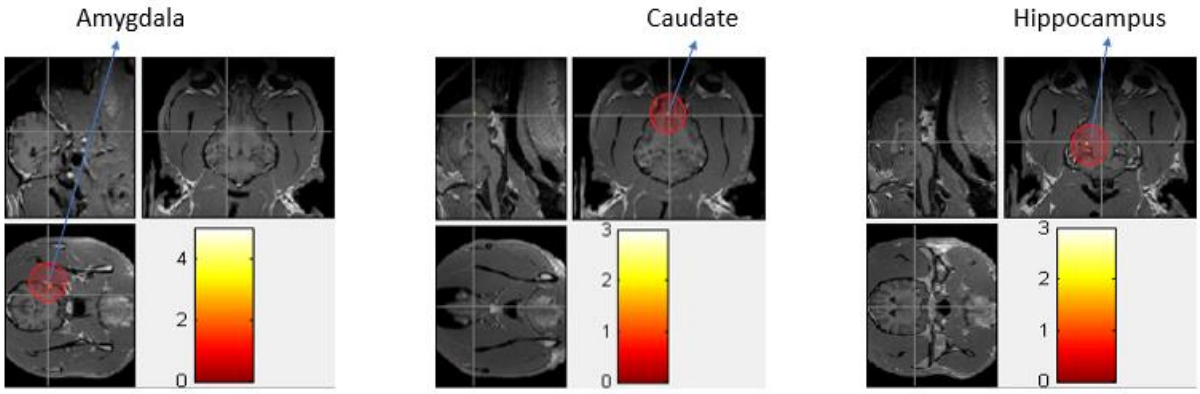

Figure 4.4: The regions with higher activation for Discriminative odor than the non discriminative odor in TP3 as compared to TP1.

Table4.2: Statistics for the regions active in TP3> TP1 for discriminative odor greater than non-discriminative odor.

S NO	Regions activated	Number of active voxels(9)	Peak T value
1	Amygdala	4	2.3129
2	Caudate	3	1.9638
3	Hippocampus	2	1.7175

4.3.2 Correlation with Behavioral measures:

The beta weights corresponding to the regions with significant activation in TP2> TP1 were obtained per subject. The difference between the behavioral scores obtained at TP2 and TP1 were calculated per subject. The beta weights and the difference in behavioral scores were

correlated and are as shown in the figure 4.5. The same is repeated for the beta weights and behavioral scores of TP3>TP1 and the correlation is as shown in the figure 4.6.

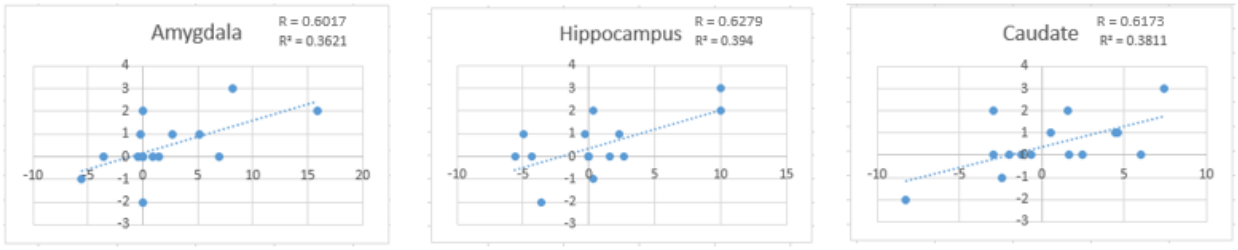

Figure 4.5: The correlation between the difference in the integrated behavioral scores of hunting, retrieving and environmental soundness of TP2 and TP1 and the average beta weights of the regions with higher activation for Discriminative odor than the Non-Discriminative odor in TP2 as compared to TP1. The behavioral scores are plotted on the X axis and the Y axis corresponds to the Beta weights.

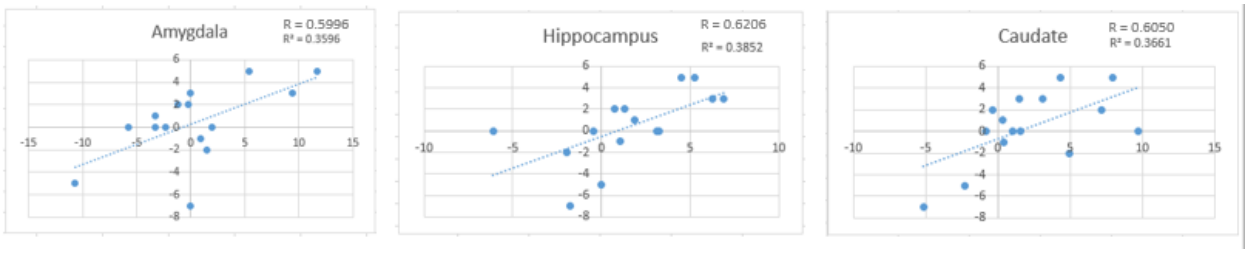

Figure 4.6: The correlation between the difference in the integrated behavioral scores of hunting, retrieving and environmental soundness of TP3 and TP1 and the average beta weights of the regions with higher activation for Discriminative odor than the Non-Discriminative odor in TP3 as compared to TP1. The behavioral scores are plotted on the X axis and the Y axis corresponds to the Beta weights

4.3.3 Activation at TP1 as a predictor for a successful detection dog:

The region with higher activation in the Successful group compared to unsuccessful group at TP1 with o significant change at TP2 or TP3 comparatively is shown in the figure 4.7. The beta weights were then obtained from this region of Olfactory bulb and fed as classifier for the logistic regression and then validated with a 4 -fold cross validation. The ROC for the same was plotted and an AUC of 0.78 was observed. The ROC are also plotted for the models using behavioral data(integrated score) at TP1 and the regions of amygdala, caudate and hippocampus obtained as the regions influenced by the detection training regime. The AUC's calculated for them are 0.5048 and 0.215 respectively. This signifies that the activation in the olfactory bulb is a preferable predictor of the success in training a dog to become a detection dog compared to its behavior.

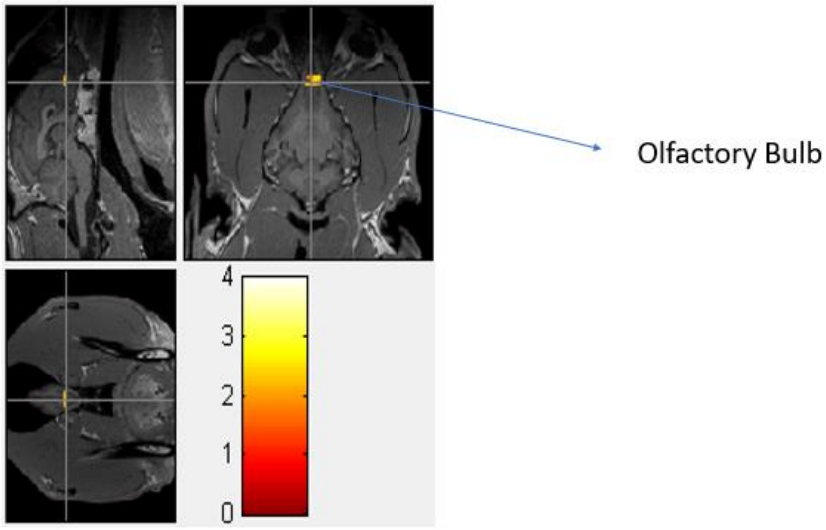

Figure 4.7: The regions with higher activation in successful group over unsuccessful group at TP1

Table 4.3: Statistics for the regions active in TP1 for success group greater than the unsuccessful group

S NO	Regions activated	Number of active voxels	Peak T value
1	Olfactory Bulb	12	2.4599

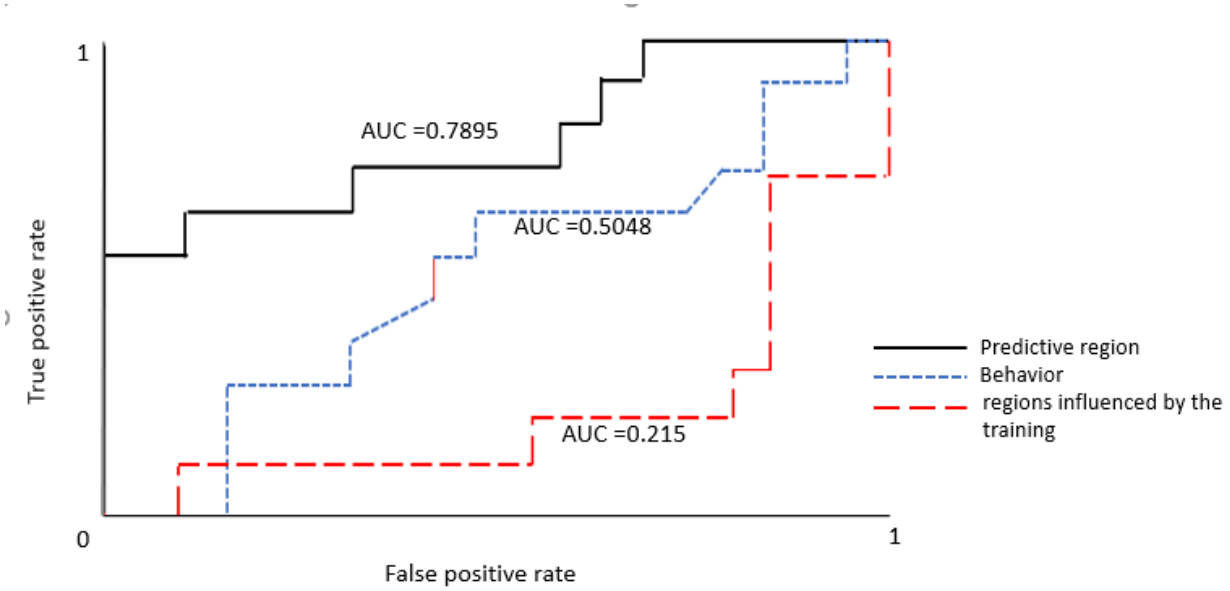

Figure 4.8: ROC plots for the classifier models to predict if a dog can be a successful detection dog using the data at TP1.

4.4 Discussion

For the past few decades studies have been conducted to understand the behavioral and neurological aspects of the dog's olfaction. To get a better know how about improving their utilization in the fields of detection In vitro (34) (93) (44) (35) and In vivo (33) studies have explored few ways of enhancing the detection capabilities in canines. This study, however is aiming at understanding the effects of odor detection training by looking at the changes in the neuronal activity of the dog brain with the help of fMRI techniques evolved in recent years.

Training of Detecting the odors had shown improvised odor recognition skills in Human beings (94). Studies have also shown that on training people with olfactory disabilities have also shown improvement in the odor detection capabilities (95) (96) (97). However these studies have all been cross sectional in nature exploring the effects before and after the training has been taken place. Similarly many studies have been conducted on the dogs where cross sectional effects of odor detection training have been explored in vivo and in vitro (30) (31) (68) (86). Apart from these it is our understanding that a training regime can be considered effective only when the capabilities acquired last far into the future.

4.4.1 Regions with higher activation because of detection training:

Looking at the results of this study the activation results of TP2>TP1 Figure 4.3 show that the regions of hippocampus, Amygdala and Caudate have an increased activation for discriminative odor as compared to non-discriminative odor post detection training. The hippocampus as we know plays a vital role in the function of learning and is especially known for its involvement in the odor detection. Hippocampus has always been an important part of olfactory memory and learning in particular (24)(98). So increased activation levels in hippocampus post training come to no surprise and are in line with our claim of the odor detection training has enhanced the detection ability of the dogs. Amygdala is known to participate involved in the processing of odor information especially in the areas of emotion, learning and memory (25). The Discriminative odorant should act as a cue to trigger a behavioral and physiological response in the canine. The increased activation in the amygdala is probably due to its involvement in the association between the odor stimulus and the response. Caudate is

a region where no known participation in odor detection is established in the literature but it is probably involved in the association of odor detection with positive expectations or rewards. Studies before have indicated caudate may reinforce learning being a part of the reward system (71)(99). The higher activation in these regions might explain the fact that the odor detection training has improved the detection capabilities and responding to it in the canines.

Observing the results of TP3> TP1 from Figure 4.4 and comparing them to TP2 > TP1 Figure 4.3 we observe that the increase in activations in the regions of amygdala, Hippocampus and Caudate has been consistent indicating that the training regime followed has lasting results resulting in Detector dogs that can perform better farther in the future.

It is also interesting to note that the activation in these regions across the time points are in positive correlation with the integrated behavioral scores.

4.4.2 Activation in Olfactory Bulb to predict the outcome of detection training:

Olfactory bulb plays an important role in the proception of odor. Many studies have already established the functionality of Olfactory bulb in humans and canines (23) (33) (37). The Olfactory bulb mainly acts as a filter but also helps performing the functions of : discriminating the odors , enhancing the sensitivity of the odor detection and lastly filtering out the background odors. So it does make sense that the activation in the olfactory bulb could be used as the predictor for a dog being a successful detection dog or not.

Chapter 5

Conclusion

Canines' olfaction capabilities are proven to be very useful to mankind over decades for various tasks of detecting explosives, people, drugs etc. However they still do not seem to be accurate due to reasons like the concentrations of the odorant in the surrounding environment. In this thesis two studies have been put forward both involving the analysis of fMRI data canines and aiming at exploring the ways of improving the detection capabilities of the dogs In the first study we have acquired the fMRI data from unrestrained awake dogs in the presence of odorants with and without zinc nanoparticles and obtained the Dynamic EC for the regions involved in Olfactory network for the contrast of OZ> O, WZ W. The results obtained have shown an increased path strengths which suggests how zinc nanoparticles can can increase the detection capability of canines .

The second study was based on the concept of A training regime can be considered successful only when the effects last over time. The fMRI and behavioral data was obtained from unrestrained awake dogs in the presence of discriminative and non discriminative odors 3 times over the time period of 8 months: TP1, Before the detection training; TP2, right after the training; TP3, a few months after the training. As we can see in the results of TP3> TP1 and TP2 > TP1 the activation is significantly higher in the same regions of the brain immediately after the training and after few months post training. This could be an indication that the training regime had a lasting positive enhancement in the odor detection capability of the dogs.

We observe that there is a clear trend, quite evidently seen from the plots shown that the dogs with higher activation for discriminative odor than non-discriminative odor in TP2 and TP3 as against TP1 are the ones with higher behavioral measures of hunting, retrieving and environmental soundness. These dogs most likely proved to be the ones with higher chances of being good detection dogs.

Looking at the analysis of predictor regions the results clearly show that the activation in the Olfactory bulb at TP1 can be a far better prediction basis than the behavioral data. This indicates that upon further refinement and replication fmri data can enable us to choose the dogs that can be successful detection dogs with a higher probability before the beginning of the training itself.

It is to be noted that the sample size though comparatively is larger than previous studies it is still small. Thus the results cannot be considered conclusive but might be indicative for further explorations. Also the effects of factors of gender, breed etc should also be explored further.

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