Introduction

Understanding the human brain and its relation to the health and function of the individual has long been a scientific pursuit. Since its first description in the Edwin Smith Surgical Papyrus, dated 17th century B.C., the brain's role in the human body has been subject to much conjecture and speculation (Robinson, 2005). Contemporary neuroscience asserts that conscious thought, the maintenance of homeostasis, sensory perception and integration, and the ability to learn are all governed by the brain (Nicholls et al., 2012). Of course, this is not an exhaustive list. Amongst its foremost capabilities is the brain's capacity to make choices and to act on those choices with autonomy (Nicholls et al., 2012). Kable and Glimcher (2009) propose the decision-making process can be characterized as appraisal; the value of available options is calculated against a set of internal criteria derived from experience and preference. Based on the situation, the option that provides the most utility to the individual is chosen (Kable and Glimcher, 2009). The purpose of the experiment outlined in this research proposal is to address the question of how this selection process is represented in the brain when value assessment is based on different reward values in different locations.

The mammalian brain has been observed carrying out at least three modes of decision making: a Pavlovian action-selection system, a habit action-selection system, and a deliberative action-selection system (van der Meer et al., 2012). Converging theories in economics, computer science, psychology, and neuroscience suggest similar conclusions with respect to deliberative decision making (Kable and Glimcher, 2009). When this system is engaged, an individual chooses the "optimal" or "best" option from an array of available possibilities (Kable and Glimcher, 2009; van der Meer et al., 2012). The option that has the greatest utility to the decision maker is ultimately chosen. If the assessment criteria for an individual – say, a rat – are known,

then behaviour can be predicted or even prompted. More importantly, assuming our rat believes a desirable reward is consistently given following specific behaviour, it will continue to demonstrate that behaviour if the benefit of the reward outweighs the cost of the physical and mental effort involved (Rigoux and Guignon, 2012). This allows for control over the movement of an animal within the confines of an experimental procedure.

There are at least two regions of the mammalian brain that provide insight into deliberative action-selection as it relates to an individual's location and the likelihood of reward. One of these regions is the ventral striatum (vStr), which is involved in the full gamut of reward-seeking behaviour (Floresco et al., 2008). The nucleus accumbens (NAc) of the vStr is associated most with risky decision-making and the selection between potential rewards based on a perceived likelihood of a cost or penalty (Floresco et al., 2008). Additionally, the vStr is implicated in reinforcement learning, or learning that results from rewarded behaviour, as long as the rewards provided are considered valuable to the individual (van der Meer and Redish, 2011). The vStr produces a signature set of frequency wave patterns called low gamma (~50 Hz) and high gamma (~80 Hz) oscillations, both of which have been observed to vary in response to rewards and can be recorded simultaneously from a freely moving organism (van der Meer and Redish, 2009). Finally, the vStr demonstrates an anticipatory ramping effect that occurs not only in populations of neurons, but also on the level of single neurons (Schultz et al., 1992). These characteristics suggest that the vStr is an ideal recording site for observing the mechanics of reward-seeking behaviour.

The second relevant brain region is the hippocampal (HC) formation, which is dedicated to identifying different settings and sub-locations within those settings as they relate to the individual (Leutgeb et al., 2005). The HC also links spatial episodes and memory, suggesting

that subjects who carry out familiar tasks in distinct settings are dependent on the HC to complete those tasks (Leutgeb et al., 2005). Furthermore, if the HC formation is lesioned, the subject becomes unable to distinguish between contexts in which only spatial features differ (Iordanova et al., 2009). Certain neurons in the HC, called place cells, are clustered in networks known as neural place cell ensembles (Leutgeb et al., 2005). These networks can be targeted in the dorsal HC for recording purposes in order to approximate their place fields, or regions that evoke rapid and frequent spiking in response to specific locations (Leutgeb et al., 2005).

Together, the vStr and HC form a relationship between reward and place (Ito et al., 2008). To establish this relationship, it was shown that fewer visits to reward sites occurred in place-oriented tasks when the bridge between these two regions was disrupted in rats (Ito et al., 2008). Lansink et al. (2012) undertook a recent study to verify that a conditioned place preference could be used to test reward-seeking behaviour and the interaction between the vStr and the HC. This research provided documentation illustrating HC neuron activity near reward sites after a cue was shown to rats, as shown in Figure 1 (Lansink et al., 2012). However, the findings in this study allow for speculation as to the association between the vStr and the HC when cues are not present. The intention of this research proposal is to present an alternative to the place task set forth by Lansink et al. (2012), one that tests the HC-vStr relationship in contexts where non-random determination of a reward site can only be discerned through spatial recollection (Lansink et al., 2012). In this case, correctly discriminating between

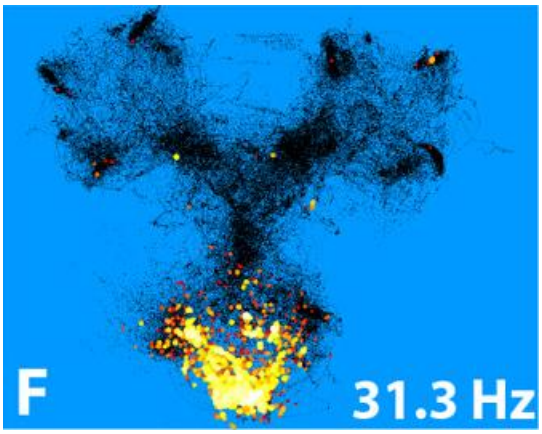

Figure 1. The firing distribution of hippocampal neurons in response to proximity to a reward port shortly after a cue. Yellow dots indicate high instantaneous firing rates of HC cells (taken from Lansink et al., 2012).

potential reward sites depends not only on a subject's awareness of its position within the environment, but also on its awareness of how its decisions are affected by past behaviour at previous reward sites.

Summary of Proposed Experiment

This experiment focuses on the use of rats as participants in a maze-like task designed to engage the HC and vStr, locations in the brain from which tetrode recordings will be taken. However, before any *in vivo* recording can begin, the rats must be comfortable with both direct human contact and regularly running the track successfully. As a result of time constraints, expenses, a lack of familiarity in training animals, and convenience, the rats used in this experiment may be inherited from Julien Catanese, a PhD student in the lab of Dr. Matthijs van der Meer. Due to the similar nature of Julien's research, these rats will be familiar with place tasks and therefore amenable to the new tasks outlined in Figure 2 and Figure 4. In the event that new rats are necessary, training would proceed as with inherited rats: an initial handling period including tickling and petting for 20 minutes daily for at least a week, followed by two to three weeks of familiarization on the task itself. No handicapped or simplified versions of the task will be used. This is done to avoid introducing confounding variables or creating a sense of unpredictability for the rats. Initially, two rats will be used as a pilot experiment, followed by a refinement period and another batch of two to four rats. Each of these rats will be expected to correctly navigate the task.

Figure 2 illustrates the layout of the track at the moment the first trial begins. As depicted by the legend in the bottom-right of Figure 2, the rat starts in the very center of the track. A block corresponding to room 1, 2, or 3 will then be removed pseudorandomly to give the rat access to

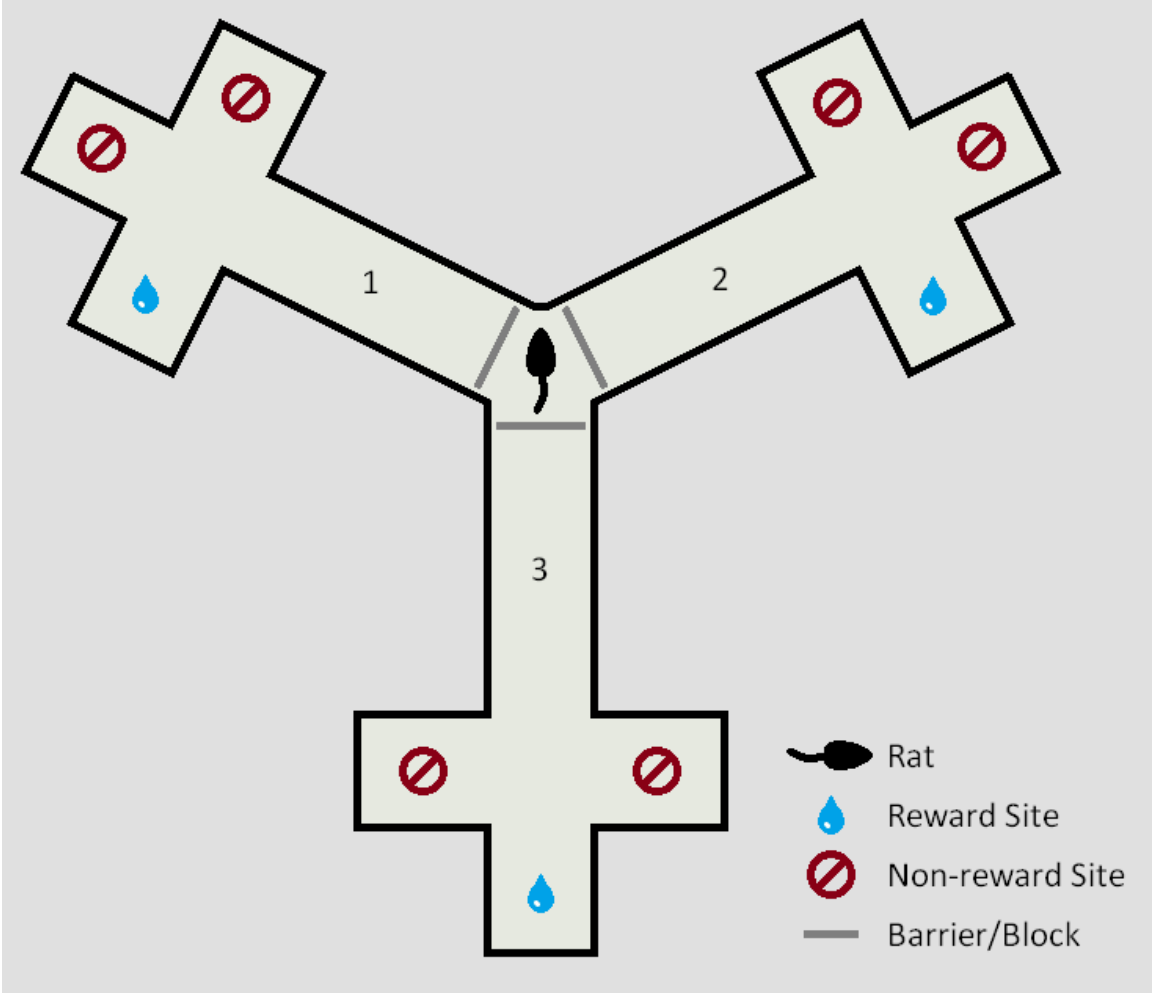

Figure 2. Floor plan of the proposed place task. The rat is displayed in its initial position. The rat will need to correctly learn which choice (left, middle, or right) will earn a reward in each location (1, 2, or 3) within the maze. Not included are the photo-beams, each of which will be placed in front of a reward site (labeled with a water droplet). Each number corresponds to a track and room with a reward site that differs from the others.

the selected track. The rat will then approach the decision point, or fork, between the potential reward sites, and if it successfully chooses the correct reward site it will be greeted with water. The water reward is administered automatically. As shown in Figure 3, if a rat trips the photo-beam sensors that section off each reward site, an automated valve will open that will allow a controlled amount of water to flow into the receptacle. Following receipt of its reward, one of the remaining two blocks on the track will also be removed pseudorandomly, providing access to a another section of the track with a new decision point. Success will be measured by the number

of correct reward site choices a rat is able to make on its first attempt at every fork. As a way to contrast this behaviour, a similar task to the one described in Figure 2 is shown in Figure 4, in which the rat merely has to turn left at every decision point in order to receive a reward. This task acts as a control, and recordings collected during trials on this setup will provide a useful comparison tool to recordings taken from rats running the task outlined in Figure 2.

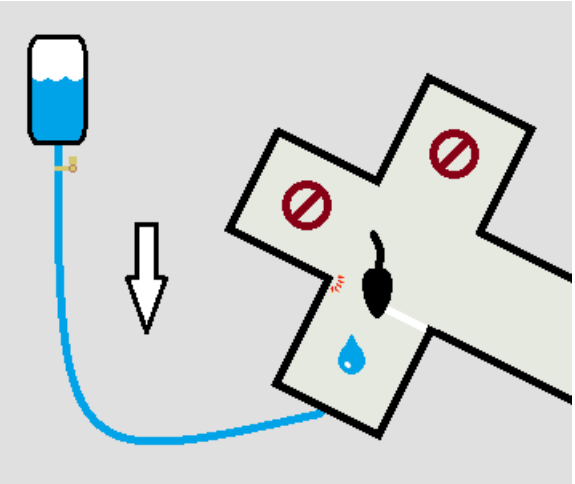

Figure 3. A demonstration of what happens when a rat passes through a photo-beam. The valve is automated, allowing gravity to bring a controlled amount of water to the reward site.

Pseudorandom block removal refers to not allowing the same block to be chosen more than three times in a row when it becomes available for removal during a trial (or three times in a row as the starting block during different sessions). This method of block removal is used as a precautionary measure designed to prevent rats from treating each new decision point in the same way as their second last reward site. Binary behaviour such as this would represent an alternation task, whereas the goal of the experiment is to force rats to use a place strategy involving the HC-vStr. Preventing a spatial alternation task from taking place also removes the confounding variable of confusion that may result from a

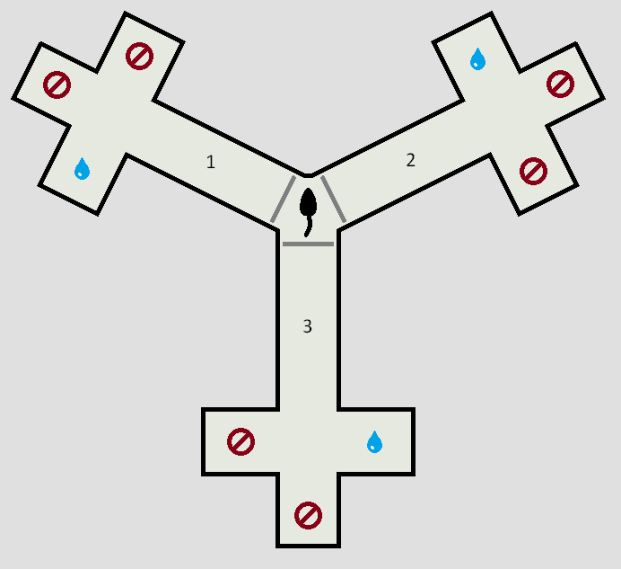

Figure 4. A control task in which rats only need to turn left in order to receive a reward. This task should elicit a different response in the HC-vStr pathway than the one proposed in Figure 2.

rat feeling cheated if the reward pattern abruptly changes. The effect of not receiving an expected reward has been shown to diminish or completely halt activity in midbrain dopamine neurons which provide input to the vStr (Ji & Shepard, 2007). It is best to avoid this phenomenon without knowing the effect it will have on a rat during its trials.

Although the proposed maze in Figure 2 differs in its structural layout from the classical T-maze used by Packard and McGaugh (1996), the most notable change in the new design (Figure 2) is that rats are expected to make choices at decision points based on past experience within the same session. While the T-maze is also a place task, the reward site never changes and rats are exposed to external environmental landmarks such as lamps, wall posters, and animal cages in order to choose the correct reward path (Packard and McGaugh, 1996). As shown in Figure 2, the design proposed in this experiment has reward sites that differ on each third of the track. Additionally, a black cloth screen will encircle the maze to block vision of its surroundings. The proposed task also strays from later place task designs, such as the one developed by Ainge et al. (2007), since it does not rely on spatial alternation and delays will not be incorporated as a variable.

Instead, the dependent variable that will be measured is the number of successful first attempts each rat has in determining a reward site. A chi-squared test will be applied to evaluate the hypothesis that rats will be making reward site choices that are deliberate and non-random. Additionally, it would be useful to contrast data from rats who failed to complete the task with those who were successful, so 100% performance is neither necessary nor desirable.

Tetrode recordings will measure alpha, low gamma, and high gamma oscillations in the vStr. Low and high gamma oscillations will be evaluated with respect to how frequently they occur prior to and following reward, while their unique, combined oscillation pattern will be

used as a landmark to verify correct tetrode placement within the vStr. A comparison between alpha and gamma oscillations should also allow for the identification of different task-solving strategies in subsequent trials (Cohen et al., 2009). The firing frequency of HC cells will also be monitored in a similar fashion to Figure 1. The expectation is that both HC and vStr neuron firing frequency will increase when the association between place and reward is made at each decision point. Additionally, different sets of neural place cell ensembles should experience increased activity prior to the rat reaching each distinct decision point (Leutgeb et al., 2005). It is expected that a simultaneous increase in the firing frequency of vStr ramp cells will occur during this time, peaking at the moment of decision (Schultz et al., 1992). If rats are not using a place strategy, there will be diminished activity in the HC when a decision point is confronted.

Results

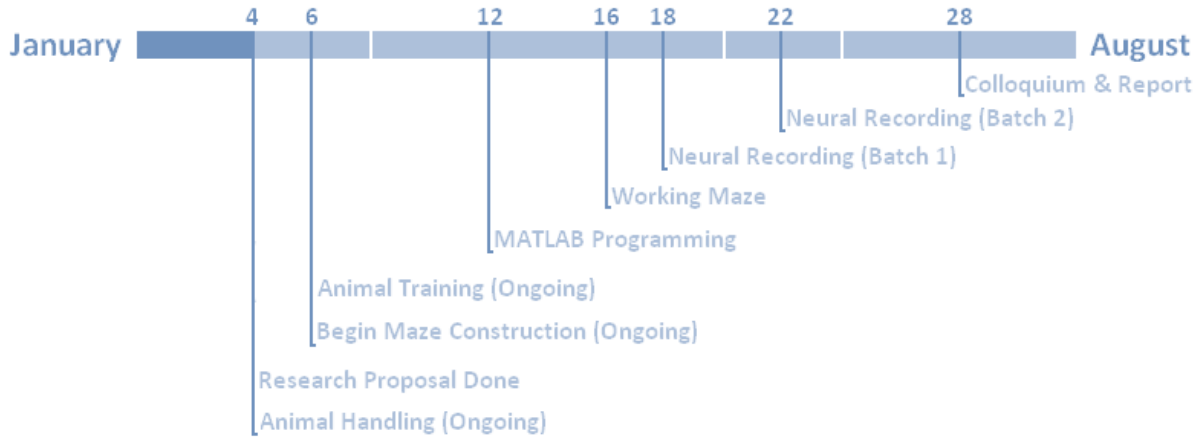

Figure 5. BIOL 499 project timeline. This is for the year 2013; numbers correspond to the sequential weeks in the year.

As it stands, no progress has been made in terms of results. Ideally, rat handling will begin in Week 4 as per the timeline included in Figure 5. Assuming the maze is constructed on schedule and there are new or inherited rats ready to run tasks by Week 16, the remainder of the experiment should come together fairly easily. While the photo-beam sensors have already been

programmed in other research projects in the van der Meer lab, the automated water delivery system has not yet been implemented. As such, a considerable amount of MATLAB programming knowledge will be required to successfully administer the water rewards. Beyond that, the largest hurdles are building the maze in a timely fashion and successfully training rats.

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