# Investigating the Contributions of Hippocampal Memory and Reward Valuation Systems to Cost-Benefit Decision Making

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## Abstract

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The hippocampus has for many decades been thought of as an essential brain region for learning about new events, often described in terms of the contexts within which particular associations, actions and outcomes occur. However, little is understood about how hippocampal context analyses impact downstream decision processes. Comparison of current events to past experiences influences our evaluation of salient stimuli, allows memory to serve a prospective organizational function, and the use of past experiences to guide current decision making processes is essential for optimal decision making. However, we know that it is rare to observe phasic neural responses to rewards, or to cues that predict rewards, by hippocampal cells in the same way that we would observe in other neurons, such as ventral tegmental area (VTA) dopamine neurons. Therefore, we sought to understand how reward and value information was represented at the level of hippocampal processing during complex decision making. To do this, we recorded hippocampal neural activity while rats performed a complex decision-making task: a maze based probability discounting task. We assessed whether the hippocampal neurons would

differentiate the context based on dynamic reward contingencies. In addition, we compared timing of these changing neural representations to VTA dopamine neural processing on the same task as they are functionally and anatomically connected to the hippocampus.

It is important to understand how brain regions in decision-relevant circuits process goal relevant stimuli differentially that can feed into memory representations of what is and isn't salient. Our first region of interest was the periaqueductal gray (PAG) as it has previously been demonstrated that it is anatomically connected to the VTA. This connection may provide a modulatory effect over the functional loop of the VTA-hippocampus by which behavior, learning, and memory mediated by the VTA are influenced by information processed in the PAG. However, it has not previously been demonstrated that the PAG encodes rewarding stimuli. Therefore, another one of the aims of this dissertation is to ascertain whether PAG neurons encode appetitive stimuli. To do this, we recorded the in-vivo activity of PAG neurons while rats performed a differential-reward working memory task.

Lastly, another goal of the current dissertation was to try to assess how these decision-making and memory functions may be affected with age. It is thought that age-related decline in separate neural systems occurs independently from each other. However, because the hippocampus is so intricately linked within a circuit that processes rewards and is essential to differentiating contexts, a functional decline in the hippocampus over time may impact decision making. Therefore, we examined the relationship between declines in spatial working memory performance and changes in risky decision making in aged rats and young controls. Additionally, we wanted to assess if observed changes in risk-based decision making behavior was linked to a functional decline in VTA neural processes on rewards and reward-relevant stimuli.

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## Chapter 1: Introduction

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The hippocampus has for many decades been thought of as an essential brain region for learning about new events, often described in terms of the contexts within which particular associations, actions and outcomes occur (Eichenbaum and Cohen, 2001; Hirsh, 1974; Kesner, 2009; O'Keefe and Nadel, 1978; Smith and Mizumori, 2006a,b). Analogous to the context-dependent spatial representation by hippocampal neurons, it was found that multiple brain areas of the decision circuitry represent decision-relevant economic information in a context-dependent fashion. This similarity suggests that there is common ground that can form the basis of interactions between decision and memory neural circuitry and operations, and that is goal-relevant context coding. Memories can be differentiated according to their duration (e.g. short and long term memory), and long term memories are often distinguished according to the type of information being held in memory (e.g. spatial, response, and emotional). Regardless of the type of information, all forms of memory are studied in terms of common operations such as those involved in the initial acquisition of information, and their subsequent consolidation, storage, retrieval, and updating. Each of these operations appear to require brain areas beyond those involved in the initial acquisition of information (e.g. spatial: hippocampus, response: striatum, and emotional: amygdala), and those brain areas are typically thought to involve one or more cortical regions. For example, it is well documented that the encoding of the emotional aspects memories involves the amygdala (e.g. Adolphs et al., 2005) but also requires intact cortical and hippocampal regions for successful encoding of the entire memory (Kensinger and Corkin,

2003). Further, the involvement of different types of memory, brain regions, and memory operations vary as a function of time, space, motivation, and past experiences. Indeed, comparison of current events to past experiences influences our evaluation of salient stimuli and allows memory to serve a prospective organizational function; the inability to use past experiences to guide future memory encoding, seen with severe retrograde amnesia, can impair the ability to encode memories into appropriate systems and guide future behavior (Klein et al., 2002).

It is worth noting that there are striking similarities in the complex nature of decisions and memory systems: there are different types of decisions (e.g. decisions relevant to actions and rewards), decision making can be broken down into its component operations (e.g., the evaluation of the costs, actual and expected outcomes, and risk when making a decision), and accurate decision-making relies on many brain areas that become differentially engaged depending on the particular operations needed to make a decision (e.g. striatum, ventral tegmental area (VTA), prefrontal cortex, orbital frontal cortex, and the amygdala). Also, similar to memory processing as described above, the involvement of different types of decisions, brain areas, and decision processes can vary across time, space, motivation, and past experiences. Further, the use of past experiences to guide current decision making processes is essential for optimal decision making. Lesions of brain regions that manage information regarding past experiences such as the medial prefrontal cortex, which is essential for recalling relevant task information to use for current decisions, impairs the retrieval of object-place and reward-location associations needed to guide decision processes (Euston et al., 2012). The complexity of memory and decision systems clearly presents a significant challenge to understanding their interactions.

An animal's understanding of the goal (or expected outcomes) of behaviors has been shown to dramatically impact cell firing in both decision and memory brain structures. However, the nature of the goal representation seems to be quite different across the two systems. Midbrain and frontal cortical responses to reward are frequently characterized in terms of their phasic, high frequency bursts when animals encounter rewards especially during initial learning (Schultz, 1997), firing to previously neutral cues that have come to predict upcoming rewards (Schultz, 1997), or ramping activity that continues until expected rewards are encountered (Howe et al., 2013). It is not uncommon for especially midbrain neural responses to upcoming or current rewards to scale relative to reward magnitude or the probability of obtaining rewards (Fiorillo et al., 2003). Also, when animals unexpectedly encounter altered or missing rewards, midbrain neurons respond with what appears as prediction error signals (Bayer and Glimcher, 2005; Hollerman and Schultz, 1998a,b; Ljungberg et al., 1992b; Mirenowcz and Schultz, 1994; Nakahara et al., 2004; Schultz and Dickenson, 2000).

In contrast to midbrain and frontal cortical neurons, it is rare to observe phasic neural responses to rewards, or to cues that predict rewards, by hippocampal cells. That is not to say, however, that hippocampal neural activity is not regulated by knowledge about learned goals. In one of the early demonstrations of place fields' sensitivity to the properties of goals, rewards were first randomly scattered in a test environment while hippocampal place fields were recorded. Place fields were observed regardless of the direction with which an animal entered the place field location (Markus et al., 1994). That is the place fields appeared 'nondirectional'. However, when only a small number of locations became associated with food, place fields became directional in that they were observed only when an animal entered the field location from one direction or another. Subsequent studies showed that hippocampal place fields tend to

be located around areas that contain information about the goal location, as well as the goal location itself (e.g. Hok et al., 2007; Hollup et al., 2001). Explicit demonstration that reward location plays a significant role in defining the context-specific properties and organization of place fields was provided by Smith and Mizumori (2006b). They showed that changing the expected goal location resulted in the reorganization (or *remapping*) of the place fields of individual neurons. Later Gill et al. (2010) showed that this goal location selective firing persisted into subsequent intertrial intervals. Thus, particular goal-defined firing patterns persisted across periods of time even when the goal is no longer present.

Additional evidence further supports the notion that hippocampal place fields are organized according to goal-defined phases of task performance. Ferbinteneau and Shapiro (2003) showed that properties of a place field depended on recent or future behaviors relative to goal acquisition. It is also now established that particular sequences of place cell activity become associated and then *replayed* after a goal-directed navigational experience (e.g. Wilson and McNaughton, 1994), or *preplayed* just prior to goal-directed navigation along familiar routes (Dragoi and Tonegawa, 2011). While it is clear that hippocampal place fields are modulated by the goals of a task, it is less clear if simply a memory-driven goal can influence hippocampal place cells to remap, i.e. represent the context as distinct. One of the goals of this current dissertation was to assess whether the hippocampus distinguished between contexts that were only defined by different reward contingencies, even though the environmental stimuli of the task and navigational paths of the animal stayed constant. That is, do hippocampal place fields distinguish between contexts that are only distinguished by changes in goal contingencies?

Place fields clearly represent multiple types of information (sensory, behavioral, goal) over time in a context-dependent manner. That is, the informational and temporal patterns are

known to become altered (or remapped) when the (internal or external) context changes (as described in Mizumori, 2008). The midbrain has long been thought to generate prediction error signals when reward outcomes vary from those expected based on past experience (Schultz and Dickenson, 2000). A comparable teaching signal is postulated to be generated by hippocampus (e.g. Mizumori, 2013). Interestingly, while remapping is commonly observed for all place fields when animals enter novel environments, changes in familiar environments result in partial remapping. In the latter case, only some 40-60% of cells (depending on the task demands) may remap when a context changes. The remaining stable place fields presumably represent constant features of a changing context (Mizumori et al., 1999). It has been hypothesized that this pattern of response illustrates one function of hippocampus and that is to detect differences in familiar and expected context features (Mizumori, 2008; 2013, 2015; Mizumori et al., 1999; 2000; Penner and Mizumori, 2012a,b), an essential operation for animals to discriminate contexts (Smith and Mizumori, 2006a). The detection of a change, or mismatch, in the expected contextual features of a situation leads to remapping, and this remapped signal can serve as a *context-prediction error signal* that might reset excitability in efferent brain areas (Mizumori, 2015; Mizumori and Jo, 2013). Since both memory and decision brain areas generate prediction error signals, understanding the nature and impact of prediction signals on memory and decision areas may lead to a better understanding of the interactions between memory and decision systems.

Since place fields are generated by the main output cells of hippocampus (the pyramidal neurons), and most hippocampal neurons are impacted by changes in expected contexts, it is reasonable to assume that hippocampus sends context-prediction error signals to efferent structures. This connection is important to understand for it likely contributes to the proper

decision about future actions that are most appropriate for a given context. The dopamine (DA) neurons of the VTA are of particular interest given its importance for encoding salience, subjective reward value, and stimuli related to rewards (Schultz et. al., 1997). Also, hippocampus seems essential for efficient reward-related locomotion and seeking as it encodes information about the temporal and environmental properties of appetitive stimuli. Further, the interaction between the hippocampus and the VTA is known to be important for context-reward associations (Luo et al., 2011). Additionally, the dorsal hippocampus has been shown to be necessary for recalling locations associated with food as lesions caused animals to reduce the number of visits to a goal location associated with food on a radial arm maze (Pothuzien et al., 2004).

Hippocampal context-prediction error signals may ultimately (via the ventral striatal-VTA path) increase the state of excitability reward responsive VTA neurons such that they become more responsive to future reward encounters. The most direct pathway for this function is via the ventral subiculum (vSub), a major output structure of the hippocampus. The vSub excites nucleus accumbens projection neurons which in turn inhibit GABAergic neurons in the ventral pallidum that project to the VTA. This releases VTA DA neurons from tonic inhibition from the ventral pallidum (Floresco et al., 2001; Lisman & Grace, 2005; Lodge & Grace, 2006). Novelty information (Knight, 1996; Lisman & Grace, 2005), which by definition involves the generation of context-prediction error signals (Mizumori, 2013), may be relayed to VTA DA neurons via this pathway. Indeed, place fields remap after a context change (Leutgeb JK et al., 2005; Mizumori et al., 1999; Smith and Mizumori, 2006a,b), and these changes do not have to be spatial in nature. Also, VTA DA reward-related firing changes after a similar context change (e.g. Puryear et al., 2010; Jo et al., 2013). Additionally, the hippocampus and VTA appear to form a functional loop wherein information is not just sent downstream from the hippocampus to

the VTA, but that subsequent processing of contextual information by the VTA is then sent back to the hippocampus for updating. Indeed, inactivation of the VTA has been shown to disrupt the stability of hippocampal place fields and increase in behavioral errors on a hippocampal-dependent working memory task (Martig and Mizumori, 2011). Another pathway linking the hippocampus and the VTA is the CA3-lateral septum-VTA pathway. This pathway is thought to be critical for enabling changes in contextual information to be integrated into the information that eventually influences motivated behaviors regulated by the VTA (Luo et al., 2011). Indeed, Luo et al showed that temporary inactivation of CA3 disrupted context-induced cocaine reinstatement (Luo et al., 2011). Thus, it is evident that the episodic-related memory information relayed from the hippocampus to the VTA is essential for guiding behaviors related to reward seeking and addiction.

The hippocampus is important for regulating motivated behaviors beyond appetitive, food-seeking, and reward-related behaviors. There is a wealth of research supporting the critical role of the hippocampus in contextual fear conditioning (Kim et al., 1993). Much of this work focuses on the functional relationship between the hippocampus and the amygdala (Richardson et al., 2004). However, the hippocampus may have an additional role in regulating defensive behaviors via the periaqueductal gray (PAG). Again, there are no direct anatomical connections between the PAG and the hippocampus. However, various studies have supported the likelihood of a functional connection between PAG and hippocampus, especially ventral hippocampus, during contextual fear conditioning. Ballesteros et al. found that when ventral hippocampus was lesioned, the dorsal PAG required a greater amount of stimulation to elicit defensive behaviors, suggesting that the hippocampus may facilitate the dorsal PAG during defensive behaviors (Ballesteros et al., 2014). In addition, the PAG is anatomically connected to the VTA, providing

both excitatory and inhibitory input onto both the GABA and DAergic neurons of the VTA (Omelchencko & Seasack, 2010; Geisler et al., 2007). This connection may provide a modulatory effect over the functional loop of the VTA-hippocampus by which behavior, learning, and memory mediated by the VTA are influenced by information processed in the PAG. For example, the PAG may transmit information to the VTA about salient negative information, such as a threat or noxious stimulus. VTA DA neurons do not respond to aversive stimuli in a homogenous manner (Brischoux et al, 2009; Matsumoto and Hikosaka, 2009) and selective inputs into VTA modulate reward and aversion differentially (Lammel et al., 2012). However, it is also possible that the PAG neurons encode rewarding stimuli, as it has not been previously investigated. Therefore, another one of the aims of this dissertation is to ascertain whether PAG neurons encode appetitive stimuli. If so, it may be an additional driver of phasic excitation to DA neurons that encode rewarding stimuli by relaying sensory information about the environment and about cues that are associated with reward to the VTA. If PAG does indeed encode appetitive stimuli, we wanted to know if reward-related responses could be modulated by the context in which rats receive rewards and their expectations therein. If so, a context-dependent response in the PAG may rely on memory information from the hippocampus.

Many of the functional relationships between the hippocampus and subcortical structures is not supported by direct anatomical connections even though a lot of evidence suggests that these disparate regions are functionally related. It is thought that the hippocampus is important for grouping information into discrete temporal packages (Buzsaki 2006, Lisman and Redish, 2009). Thus, the hippocampus may coordinate with these areas, in the absence of direct anatomical projections, via functional coupling through oscillatory mechanisms, i.e. phase locking these other regions to hippocampal theta during critical times. These critical times

would be defined as times during which extra-hippocampal structures need working memory/contextual input to select the most appropriate behavior for the given situation. Indeed, the hippocampus has been shown to have synchronous oscillatory activity with the VTA and medial prefrontal cortex in the 4 Hz and theta frequencies during a working memory task, suggesting that oscillatory mechanisms may be important for coordinating information processing between areas to support various cognitive functions such as working memory (Fujisawa and Buzsaki, 2011). Although much work remains to be done to parse out the particular contributions of the hippocampus to each one of these subcortical regions, it appears that the hippocampus' ability to discriminate changes in context, regardless of the modality of change, is an important part of regulating many motivated behaviors. Additionally, it appears that the relationship between the hippocampus and the aforementioned subcortical structures creates a functional loop essential for the creation of new episodic memories related to various motivated behaviors. Indeed, many of the above mentioned structures, in addition to receiving contextual information from the hippocampus, also send information to the hippocampus.

Lastly, the other goal of the current dissertation was to try to assess how these decision-making and memory functions may be affected with age. Normal aging is often accompanied by noticeable changes in behavior, such decision making and memory functions, even in the absence of neurodegenerative disease. These changes are not due to neuron death but instead more subtle physiological changes. Neurocognitive processes that are mediated by the hippocampus, such as declarative memory and pattern separation, and the dorsolateral prefrontal cortex (PFC), such as cognitive flexibility and working memory, are the most susceptible to age-related changes (Morrison & Baxter, 2012). Age related decline of hippocampal function has been vigorously studied in rats using spatial memory tasks such as the Morris water maze

(Gallagher, Stocker, & Koh, 2011). These tests reveal aged rats consistently show impairment on hippocampal dependent tasks compared to young rats (Gallagher, Stocker, & Koh, 2011). Indeed, in vivo investigation into hippocampal function reveals that place cells are less likely to update in new environments, and this decrease in adaptability is correlated with deficits in spatial memory (Wilson et. al., 2004). It is thought that decline in separate neural systems occurs independently from each other; thus, declines in PFC function does not necessarily imply deficits in hippocampal function and vice versa (Fletcher & Rapp, 2013). However, because the hippocampus is so intricately linked within a circuit that processes rewards and is essential to differentiating contexts, a functional decline in the hippocampus over time may impact decision making. Therefore, we examined the relationship between declines in spatial working memory performance and changes in risky decision making in aged rats and young controls.

## Chapter 2: Hippocampal neural activity reflects the economy of choices during goal-directed navigation

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## INTRODUCTION

The critical role of the hippocampus in episodic memory (Tulving, 2002) likely reflects pattern separation processes (Yassa and Stark, 2011) which include computations that distinguish contexts or situations by comparing their predicted features relative to those actually experienced (e.g., Mizumori et al., 1999; Smith and Mizumori, 2006; Mizumori, 2008; Duncan et al., 2012; Penner and Mizumori, 2012; Mizumori and Tryon, 2015). In support of this view, location-selective firing (i.e. *place fields*) of many hippocampal *place cells* are altered when contextual features of a familiar situation change, features such as external multisensory information, behavioral requirements, memory demands, or reward location (Smith and Mizumori, 2006; Mizumori, 2008; O'Keefe, 1976; Muller and Kubie, 1987; Fyhn et al., 2002; Leutgeb et al., 2005a and b). Further, when rats are explicitly trained on a mismatch detection task, it has been shown that hippocampal neurons fire differently on match and mismatch trials (Manns et al., 2007).

Presumably, hippocampal neural activity during goal-directed navigation reflects decisions based on an analysis of context information. These decisions should optimize the conditions and likelihood that a goal will be achieved. The types of goal information that could guide such choices and decisions is not well understood. Knowledge of the location of a goal has been

shown to bias the distribution of place fields around the goal location (Hollup et al., 2001; Lee et al., 2006). When explicitly trained to do so, rats behaviorally and neurally distinguish contexts based on their different goal locations even when other sensory, behavioral, and motivational aspects of a task are held constant (Markus et al., 1995; Smith and Mizumori, 2006). Such goal location-defined neural firing patterns continue to be observed when rats are removed from the maze during the intertrial interval (Gill et al., 2011). That knowledge of the location of a current goal can exert a powerful guiding influence on the organization of neural activity in hippocampus has also been demonstrated by findings that the place fields, and their experience-dependent sequential activation, depending on recent or future goal-directed trajectories (Wood et al., 2000; Louie and Wilson, 2001; Lee and Wilson, 2002; Foster and Wilson, 2006; Diba and Buzsaki, 2007; Johnson and Redish, 2007; Karlsson and Frank, 2009; Lisman and Redish, 2009; Dragoi and Tonegawa, 2011; Ferbinteanu et al., 2011; Pfeiffer and Foster, 2013; Cei et al., 2014; Wikenheiser and Redish, 2015).

Recordings from midbrain dopamine neurons of the ventral tegmental area (VTA) show that neurons signal other aspects of a goal in addition to its location. For instance, dopamine cells signal the novel presentation of rewards, as well as the cues that predict future rewards (Schultz et al., 1997; Fiorillo et al., 2003). These reward-related responses are modulated by the learned economic conditions of reward acquisition such as the probability that rewards will be delivered at particular reward sites. This study sought to determine whether similar characteristics of a goal (other than its location) drive the characteristics of spatial representation in hippocampus. Also of interest was whether theta in the local field potentials (LFPs) respond to different types of goal information since theta appears important for spatial decision making (Belchior et al., 2014) and spatial memory (Winson, 1978; Mizumori et al., 1989).

To assess whether hippocampal neural processing reflects goal information other than its location, we developed a probability discounting maze-based task in which hippocampal activity was recorded as rats made choices that were based on their understanding of the probabilistic nature of a goal that is found in a constant location. Notably, there was no right or wrong choice; rather we assessed biases in choices that varied with changes in econometric factors of interest such as the probability of an outcome, agency (i.e. whether rats made free choices or were forced to make a specific choice), and magnitude of expected rewards. These features systematically varied within a single recording session so that the same neural (place field and LFP) responses could be related to changes in decision biases. For comparison, a follow up study included recordings from VTA dopamine neurons as a different set of rats performed the same probability discounting task used for the hippocampal recordings.

## MATERIALS AND METHODS

### *Subjects.*

Three (for hippocampal recordings) and two (for VTA recordings) male Long–Evans rats (350–420 g; Simonsen Laboratories) were housed individually in Plexiglas cages. The rats were maintained on a 12 h light/dark cycle (lights on at 7:00 A.M.) and all behavioral experiments were performed during the light phase. Each rat was allowed access to water *ad libitum* and food-deprived to 80% of its *ad libitum* feeding weight. All animal care and use were conducted in accordance with University of Washington's Institutional Animal Care and Use Committee guidelines.

### *Probability discounting maze task.*

Rats were trained to run on an elevated loop maze (130cm long x 86cm wide x 80cm high; **Fig. 2.1A**; developed with the assistance of Cowen, S. (http://cowen.faculty.arizona.edu/). As part of pretraining, rats were first habituated to the maze and maze environment. To habituate rats to the maze, rats were allowed to freely forage for sugar pellets (45mg, TestDiet) scattered around the maze. Once they consistently consumed the sugar pellets and traversed the length of the maze, pretraining on the probability task began. The beginning of pretraining consisted of a simplified version of the task during which one door was open at a time. Passage through each door was associated with a 50% probability of receiving one sugar pellet. Once rats were reliably running the maze, which took an average of 10.5 pretraining sessions, their side preference was assessed, and the large risky option was assigned to either their preferred side or their non-preferred side in a counterbalanced manner. At this time, training on the probability discounting maze task began.

The fully automated probability discounting task was modeled after one used with operant chambers (St. Onge and Floresco, 2009). Each session started when the rat was placed on the maze. After a two second delay, the start door opened and the rat turned right to arrive at the decision platform. One door was associated with the large reward/risky option (defined below), the other the small reward/certain option (also defined below), and this was held constant for each rat throughout training but counterbalanced across rats. A sensor was located on each of the doors to track the door choice of the rat. After the rat passed through one of the "decision" doors (either the large reward/risky choice or the small reward/certain choice), it triggered another sensor that closed the decision doors preventing the rat from turning back. As the rat continued down the maze, passing another sensor triggered the delivery of the food reward so that a pellet(s) would be present in the food cup once the rat arrived there. An audible click signaled

the delivery of each food pellet (80209 pellet dispenser, Campden Instruments Ltd., A Lafayette Instrument Company). Choice of the small reward/certain option always delivered one pellet (one click) with 100% probability; choice of the large reward/risky option delivered four pellets (four clicks) but with varying probability. A sensor was located at the food cup to record precise arrival at the reward location. After consuming the reward, the rat had to return to the start sensor to initiate the next trial.

Each recording session assessed choice behavior and neural activity across 64 trials that were separated into 4 probability blocks of 16 trials each (**Fig. 2.1B**). The probability blocks of trials delivered a large reward with 100%, 50%, 25% or 12.5% probability (presented in descending order) when rats chose the large reward/risky option. During each probability block, the first 8 trials were forced choice trials (in which only one option was available) while the next 8 trials were free choice trials (in which both doors were open). The forced choice trials informed the rats as to the current probability of obtaining a large reward when choosing the large reward/risky option. Selection of small reward/certain option always resulted in a small reward 100% of the time. Thus, blocks 1 (forced choice) and 2 (free choice) were associated with 100% probability of receiving the large reward, 3 (forced) and 4 (free) with 50% probability, 5 (forced) and 6 (free) with 25% probability, and 7 (forced) and 8 (free) with 12.5% probability if the large reward/risky option was chosen. After rats were consistently completing all trials in a session across multiple days and displayed discounting behavior, they underwent a surgical procedure for the implantation of recording electrodes. Each rat ran an average of 13.5 probability discounting sessions before surgery.

***Surgery.***

Each rat was placed in an induction chamber and deeply anesthetized under isoflurane (4% mix with oxygen at a flow rate of 1 L/min). While unconscious, the animal was placed in a stereotaxic instrument (David Kopf Instruments) and anesthesia was maintained throughout surgery by isoflurane (1 ~ 2.5%) delivered via a nosecone. The skull was exposed and adjusted to place bregma and lambda on the same horizontal plane. A microdrive array (described below) was unilaterally implanted so that the tetrodes could be placed dorsal to the hippocampus (3.6-3.7 mm posterior from Bregma, 3.5 mm lateral to the midsaggital suture, and 2.0 mm ventral from the dural surface) or VTA (5.3 - 6.0 mm posterior from Bregma, 1.0 mm lateral to the midsaggital suture, and -7.0 mm ventral from the dural surface). The drive assembly was secured in place with anchoring screws and dental cement. After surgery, an analgesic (Metacam, 1mg/kg) and an antibiotic (Baytril, 5mg/kg) were administered. Rats were allowed to recover for 7 days, during which they were fed *ad libitum* and handled daily.

***Single-unit recording and postsurgical procedures.***

Recording tetrodes were constructed from 20 μm lacquer-coated tungsten wires (California Fine Wire) and mounted on an array of twelve independently adjustable microdrives (Harlan 12 Drive, Neuralynx). Tetrode tips were gold-plated to reduce impedance to 0.2–0.4 MΩ (tested at 1 kHz). After a week of recovery from surgery, rats were returned to a food-restricted diet and spontaneous neural activity in the hippocampus was monitored as follows on a daily basis: the electrode interface board of the microdrives was connected to preamplifiers, and the outputs were transferred to a Cheetah digital data acquisition system (Neuralynx). Hippocampal single unit (0.6 – 6.0 kHz) and LFP (0.3 – 300 Hz) signals were filtered then digitized at 16 kHz. For VTA neural recordings the low end of the filtering window was expanded to 0.1 Hz. Neuronal spikes were recorded for 2 ms after the voltage deflection exceeded a predetermined threshold at

500–7000× amplification. At the end of each recording session, tetrodes were lowered in 40 μm increments, up to 160 μm per day, to target new units for the following day. Experimental sessions continued until tetrodes passed through the hippocampus (or VTA) based on the distance traveled from the brain surface. A video camera mounted on the ceiling tracked infrared LED signals attached to the preamplifier and subsequent position data were fed to the acquisition system.

Once clearly isolated and stable units were found, the rats were recorded during a probability discounting session. The session began by placing the rat on the start segment of the maze. Two sec after the start sensor was triggered, the start door opened and the session continued until all 64 trials were completed. The sensor-driven opening and closing of maze doors was controlled automatically by a custom program (Z-basic). A timestamp of each triggered sensor was fed into the Neuralynx system so that they can be incorporated into future spike and video analyses

***Histology.***

After the completion of all recording sessions, tetrode locations were verified histologically. Rats were deeply anesthetized under 4% isoflurane. The final position of each tetrode was marked by passing a 15 μA current through a subset of the tetrode tips for 15 s. Then, the animals were given an overdose of sodium pentobarbital and transcardially perfused with 0.9% saline and a 10% formaldehyde solution. Brains were stored in a 10% formalin–30% sucrose solution at 4°C for 72 h. The brains were frozen, and then cut in coronal sections (40 μm) on a freezing microtome. The sections were then mounted on gelatin-coated slides, stained with Cresyl violet, and examined under light microscopy. Only cells verified to be recorded in hippocampus, or the VTA in the follow up study, were included in the data analysis.

***Data analysis.***

Hippocampal and VTA single units were isolated using an Offline Sorter (Plexon). Various waveform features, such as the relative peak, valley, width, and principle component, were compared across multiple units simultaneously recorded from four wires of a tetrode. Only units showing good recording stability across blocks were included. Further analysis of the sorted units was performed with custom Matlab software (Mathworks).

Putative hippocampal pyramidal (place) neurons were initially identified, as in prior studies, according to mean firing rate (less than 10 Hz) and exhibition of high-frequency complex-spike bursts as revealed in autorcorrelograms and interspike interval histograms. Data from cells that did not exhibit complex spike bursts were not included in subsequent analyses.

*Place Field Identification and Characterization:* Maps were constructed for the spike and occupancy analysis by binning pixels using square bins ~2.5 cm. The spike map and occupancy map were separately smoothed using 2D convolution with a nine bin discrete Gaussian kernel (sigma ~0.8493) centered on each bin. The rate map was generated by dividing each pixel of the smoothed spike map by each pixel in the smoothed occupancy map. Place fields were located by constructing a binary rate map from the smoothed rate map using a threshold of 20% of the peak session smooth rate. In addition, the block conditions being compared had to show place fields with a peak rate of at least 1 Hz, and the primary place field was defined as the largest field that had an infield rate greater than or equal to 20% of the peak rate. Given the location of each field, values for the number of spikes within a field were determined using the smoothed rate maps. Similar criteria have been used in previous studies (e.g. Smith et al., 2006, 2012; Gill et al., 2011; Martig et al., 2011), and 97% of the cells analyzed showed mean firing rates less than 3 Hz, with the average rate less than 0.8 Hz. Therefore, the likelihood of including interneurons in

our sample is very low. Common metrics of place field stability (information content, reliability and spatial specificity) were calculated as previously published (Smith and Mizumori, 2006; Skaggs et al., 1993).

*Stability of place field organization:* The stability of location selective firing of place cells was calculated only for trial blocks that had a primary field that met the following criteria: number of infield spikes $\geq$ 1; area of the field $\leq$30% of the total visited pixels for that block; infield/outfield rate ratio score $\geq$ 0.3 (which corresponds to the infield rate being twice that of that outfield rate); reliability $\geq$ 0.3; mean session rate $\geq$ 0.1 Hz and <10Hz.

*Linearized Maze:* The maze was linearized by using an ellipse to fit the path of the maze, which is roughly ellipsoidal. The ellipse was then segmented into 100 equal length segments and assigned a corresponding bin number. Each position from the video was assigned to the linear bin that was closest, as defined by the Euclidean distance. In order to correct for small deviations in the rat's position, the linear bins were smoothed using a lowess differentiation filter.

*LFP Analysis:* Values for velocity, firing rate, and power were only included when the rat's position monotonically increased with respect to the bin numbers (i.e. they were moving forward). Values were averaged when successive frames had repeating values for a bin. Values were also averaged across blocks if needed for a particular analysis. Power was calculated using the multitaper Fourier analysis, mtspecgramc, from the Chronux toolbox, using a 500 ms window with a 50 ms step. The resulting spectrogram was filtered and averaged over each frequency band and converted to dB using the relation 10*log10(μV^2)/Hz). The theta frequency band used in this analysis was 6-10 Hz.

*Phase Precession:* Theta phase precession refers to successive spikes of a place cell bursts that occur at progressively earlier phases of a theta cycle (O'Keefe and Recce, 1993). We examined whether the degree of phase precession varied as a function of the econometric and behavioral factors described above for place fields. Continuous recordings of local field potential (extracellular potential) were filtered for components in the theta frequency range (6-10 Hz) using a Chebyshev Type 2 bandpass filter. The Hilbert transform was applied to get instantaneous phase over time. Distance through the field was measured by calculating the length of the rat's path until the time when a spike occurs. Distance through the field for each pass was normalized for the total length of that pass through the field. Each pass had to have a minimum of 5 spikes in at least 4 theta cycles, a mean velocity of at least 3 cm/s, and a maximum interspike interval of 1 second (Schlesiger et al., 2015). Additionally the place fields had to have reliability and specificity scores greater than 0.3, an area less than a third of all visited pixels (in the block), and firing rates between 0.1 Hz and 10 Hz. Phases for each spike were calculated by matching the closest timestamp of the spike to the LFP timestamps and using the phase as calculated from the Hilbert transformation. Data from multiple passes in each block were pooled into one data set then analyzed together; single pass data were not analyzed. Theta phase precession was calculated as the slope of spike phase versus normalized distance into the field. A circular-linear regression method was applied (Kempter et al., 2012).

*VTA data analysis.* VTA data were collected from two rats over 23 sessions. Putative dopamine and non-dopamine cells were classified using a cluster analysis developed to identify dopamine cells in the rodent VTA (Jo et al., 2013; Roesch et al., 2007; Jin and Costa, 2010) as previously described (see Jo et al., 2013). Briefly, using the tetrode channel with the largest peak-to-valley amplitude, two basic characteristics of the average spike waveform were determined for each

cell: (1) the half time of the spike duration (i.e., measured between the first valley and the next peak); and (2) the amplitude ratio of the first positive peak and negative valley in a waveform [(n - p)/(n+ p), with n as the first negative valley and p as the first positive peak]. Then, a scatter plot including all VTA cells was constructed (**Fig. 2.12B**). A cluster showing broad half duration and low amplitude ratio was putatively classified as dopaminergic.

*Statistical analysis.* A multiple linear regression was performed on every cell to determine if agency, probability, or outcome could significantly predict changes in primary place field location and/or rate. To do this, the location and mean rate of the primary place field for each cell was determined for every trial in a session. If the primary place field did not meet the above criteria, the trial was assigned as having no field. Then, the econometric variables (i.e. agency, probability, and outcome) were assigned as the predictor variables in the regression to predict either location or rate (regressions were ran separately for location and rate). All regressions were determined using SPSS version 22, IBM. $\alpha$ was set at $p \leq 0.05$.

To assess whether probability of reward, choice outcome and/or agency similarly contributed to the changes observed in theta power throughout the probability discounting task, the maze was divided into eight discrete regions (defined in the results section) that were associated with different task phases. The power for each tetrode during each trial was determined and a multiple linear regression analysis was run for each region with agency, outcome, probability, and velocity as predictors (SPSS version 22, IBM). $\alpha$ was set at $p \leq 0.05$.

To determine whether or not place fields were distributed in a biased fashion across the maze, the frequencies of place fields in the same eight regions used for the LFP analysis were assessed using the Chi square test. The null hypothesis was that the place fields were evenly distributed

across the different regions of the maze, adjusted for the size of each of the eight regions. Place fields distributions were considered unequal with α set at 0.05. Additionally, to specifically test if there were more place fields in the feeder region of the maze, a binomial test was performed comparing the observed and expected number of place fields in the feeder region and the rest of the maze. Expected numbers of place fields were based on the size of the region (i.e. the feeder region was 22% of the maze area so 22% of place fields would be expected there). $p \leq 0.05$ indicates there were significantly more or less fields in the feeder region than expected.

**RESULTS**

Probability discounting behavior on a maze: A total of 146 sessions were recorded (36.5 sessions per rat on average, ranging from 20 to 54 sessions) from 3 rats. In 89 of 146 recording sessions, rats exhibited probability discounting behavior, which was defined as the choice for the large risky reward during the highest probability (100%) always being at least 10% greater than the choice for the lowest probability (12.5%) Indeed, there was a significant main effect of probability on choice of the large reward (repeated measures ANOVA, $F(3, 88) = 111.5$, $p < 0.001$), and choice for the large reward during 100% probability was greater than the three other probabilities (Bonferroni post-hoc test for pairwise comparisons, $p < 0.05$). **Figure 2.1C** shows that during these sessions rats preferred the risky (large reward) option when the probability of receiving a large reward was high. As the probability of receiving the reward declined, so did the rats' preference for choosing the risky option.

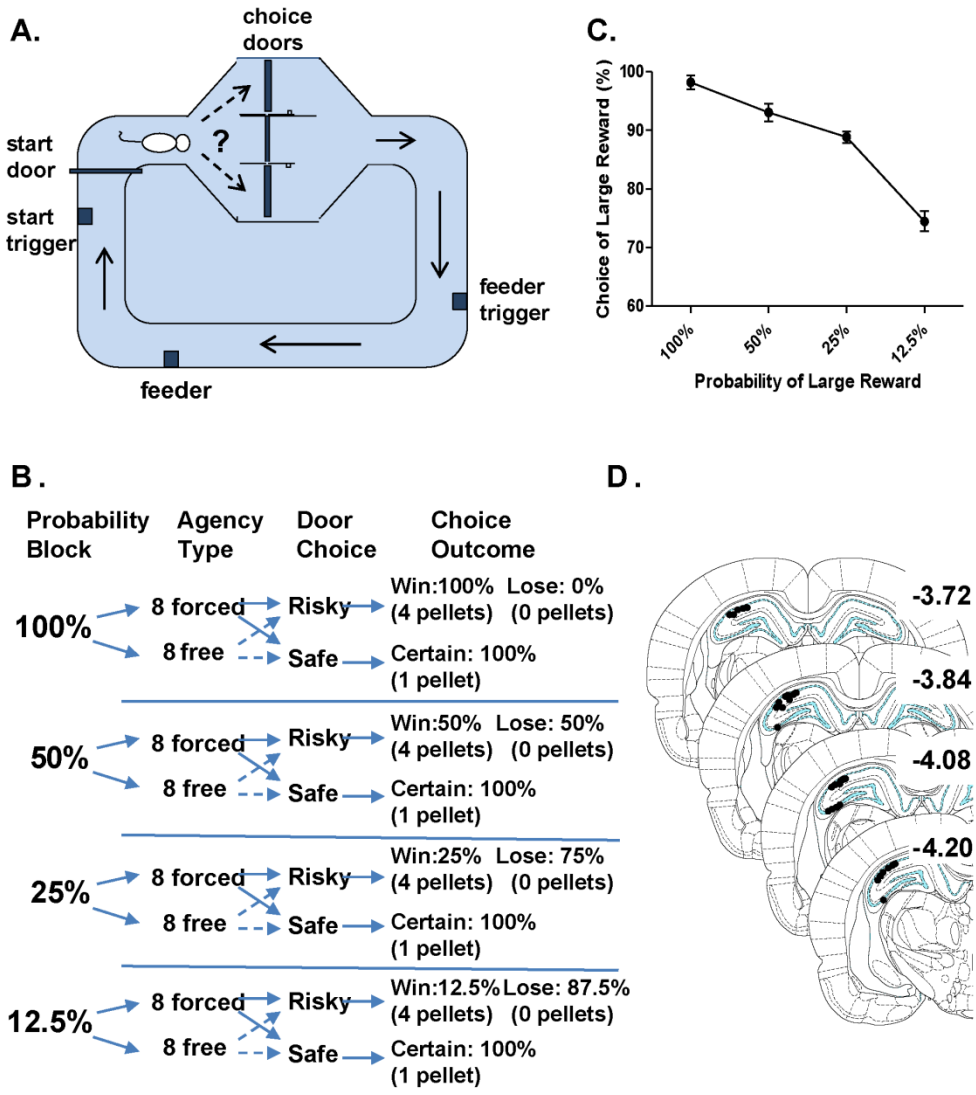
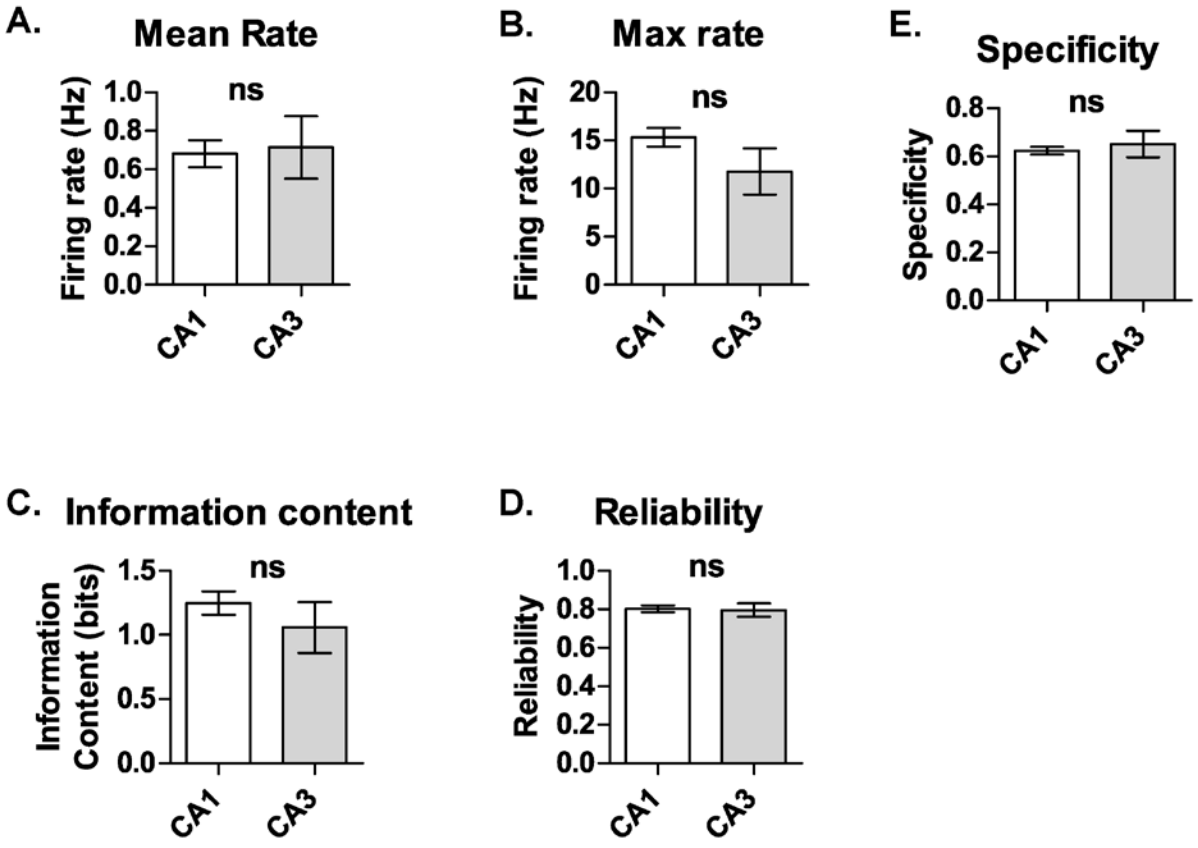



**Figure 2.1. A.** Schematic of the loop maze used to assess probability discounting behavior. Movement sensors were located at key locations on the maze (e.g., start door, door exit, feeder

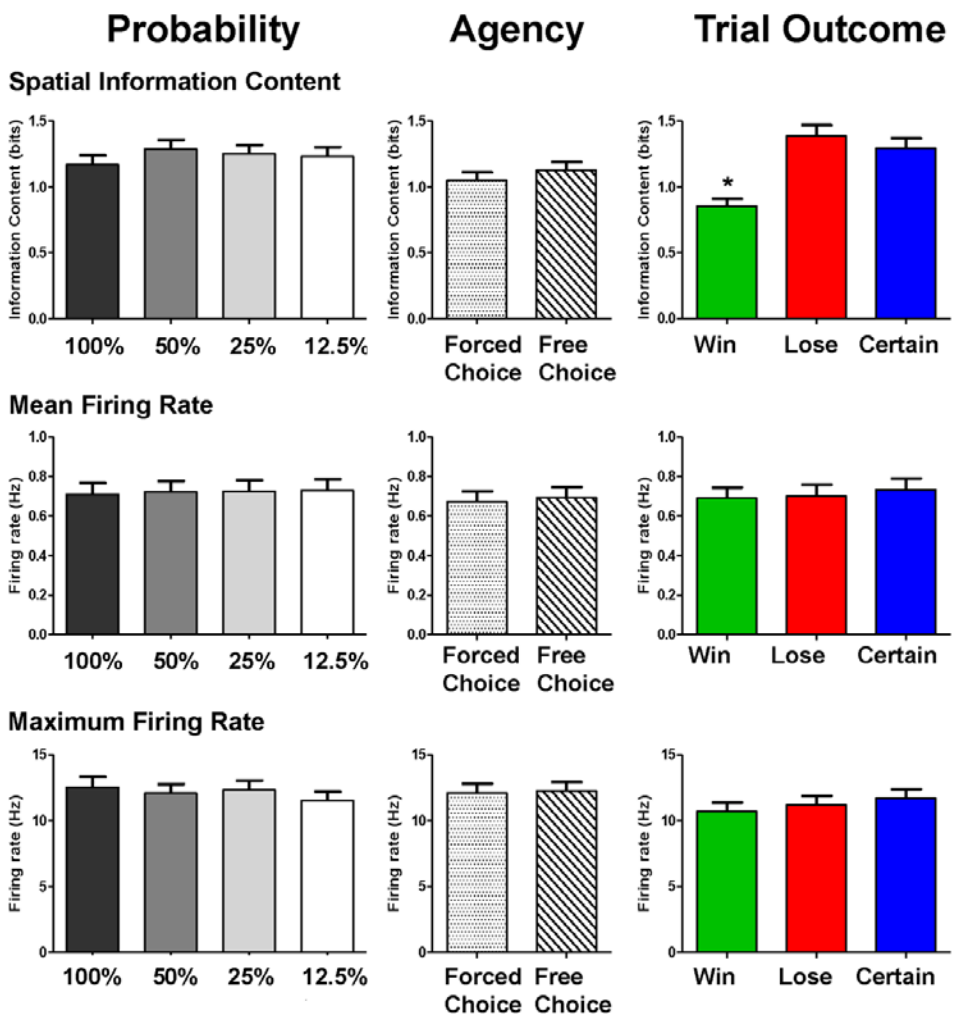
trigger, feeder). **B.** Each session assessed choice behavior and neural activity across 64 trials that were separated into 4 probability blocks of 16 trials each. The different probability blocks of trials denoted that a large reward would be delivered with 100%, 50%, 25% or 12.5% probability (presented in descending order) when rats chose the risky option. During each probability block, the first 8 trials were forced choice trials (in which only one door was open) while the next 8 trials were free choice trials (in which both doors were open). The forced choice trials informed the rats as to the current probability of large reward. Selection of the safe, certain option always resulted in a small reward 100% of the time. **C.** A total of 146 sessions were recorded (36.5 sessions per rat on average, ranging from 20 to 54 sessions) from 3 rats. In 89 sessions, rats displayed clear discounting behavior (error bars represent SEM). Data shown were taken only from sessions during which rats exhibited discounting behavior. **D.** Histological reconstruction of the hippocampal recording sites show that most of the recordings occurred in dorsal CA1 with a smaller number in dorsal CA3. Since the responses of the two populations of place cells were similar, their data were combined for analysis.

Place fields remain spatially selective despite changes in the probability of reward or agency:

Out of 909 recorded hippocampal neurons, 573 were identified as putative place cells according to standard criteria (Smith and Mizumori, 2006). Of these, 424 units were recorded from CA1 and 149 from CA3. These groups were combined for analyses since no differences were observed on any measure (**Fig. 2.2**). Place cells showed strong location-specific firing regardless of changes in expected reward probability (i.e., 100%, 50%, 25%, or 12.5%), agency (i.e., forced choice or free choice), or choice outcome (i.e., win or lose), (**Fig. 2.3**). The whole session mean firing rates and the maximum firing rate within place fields did not significantly vary as a function of expected reward probability, agency, or choice outcome (all p's > .05). Generally, information content also did not change as a function of reward probability or agency with the exception of win trials, which had significantly lower information content compared to the other outcomes (1-way ANOVA, $F(2,748) = 15.58$, $p < 0.0001$). This pattern may reflect the tendency of place fields to be located around the goal location after the feeder signal confirms to the rat that food will be delivered on a win trial (see below).




**<u>Figure 2.2.</u> Place field properties were not different between CA1 and CA3 cell populations.** When comparing **A.** mean rate, **B.** max rate, **C.** information content, **D.** reliability, and **E.** specificity values between CA1 and CA3 neurons, none of the values were significantly different from each other (independent t tests, all *p's* > .01, $\alpha = 0.01$; Bonferroni correction for multiple comparisons). NS = not significant.




**<u>Figure 2.3.</u> Place field properties did not change as a function of varying reward probability, agency and choice outcome.** *Top row*: With one exception, spatial information content (mean <u>+</u> SEM) was similar across the different probability blocks and agency conditions (all *p's* > .05). The right panel shows that information content was significantly lower during win

trials relative to lose and certain trials ($F = 20.33$, $p < .0001$). *Middle row*: The average mean firing rate of place cells did not change as a function of probability of reward, choice agency, or choice outcome (all $p$'s $> .05$). *Bottom row*: The average maximum firing rate within place fields did not change as a function of probability of reward, choice agency, or choice outcome (all $p$'s $> .05$). These data show that (with one exception) standard measures of the integrity of place fields did not change during experimental manipulations.

The locations of place fields were biased to occur close to the goal location when rewards are expected, but the degree of bias depended on the expected probability of receiving rewards: When considering the overall recording session, place fields were observed across the entire length of the (linearized) maze. A differential distribution of place fields became apparent, however, when trials were sorted according to reward probability, agency and trial outcome (**Fig. 2.4; Table 1**). Significantly more place fields were found around the reward location for the 12.5% win trials in both forced and free choice conditions (binomial test, $p < 0.001$; **Table 2)**. While not significant, place fields also tended to accumulate around the reward site for free-choice certain trials while place fields were not preferentially found near the reward location on lose trials in any condition. This finding is consistent with prior reports that place fields tend to be found near significant locations such as reward and goal locations (e.g., Hollup et al., 2001). Specifically, these data show that the learned probability of receiving rewards and agency are important factors that determine goal-related place cell activity. This preference for place field locations emerged prior to arrival at the feeder, indicating that the auditory cue that signaled impending reward was at least in part responsible for the subsequent place field reorganization. The presence of the cue per se, however, could not have been the only factor initiating this response as the bias for place fields to be located near the reward location was most striking during low reward probability (win) trials. In sum, the scaling of the redistribution of place fields relative to the probability conditions of trials was observed only for win trials (under both forced and free choice conditions). In contrast, goal location preferences of place fields were observed only when rats freely chose the safe, certain option regardless of the probability condition.

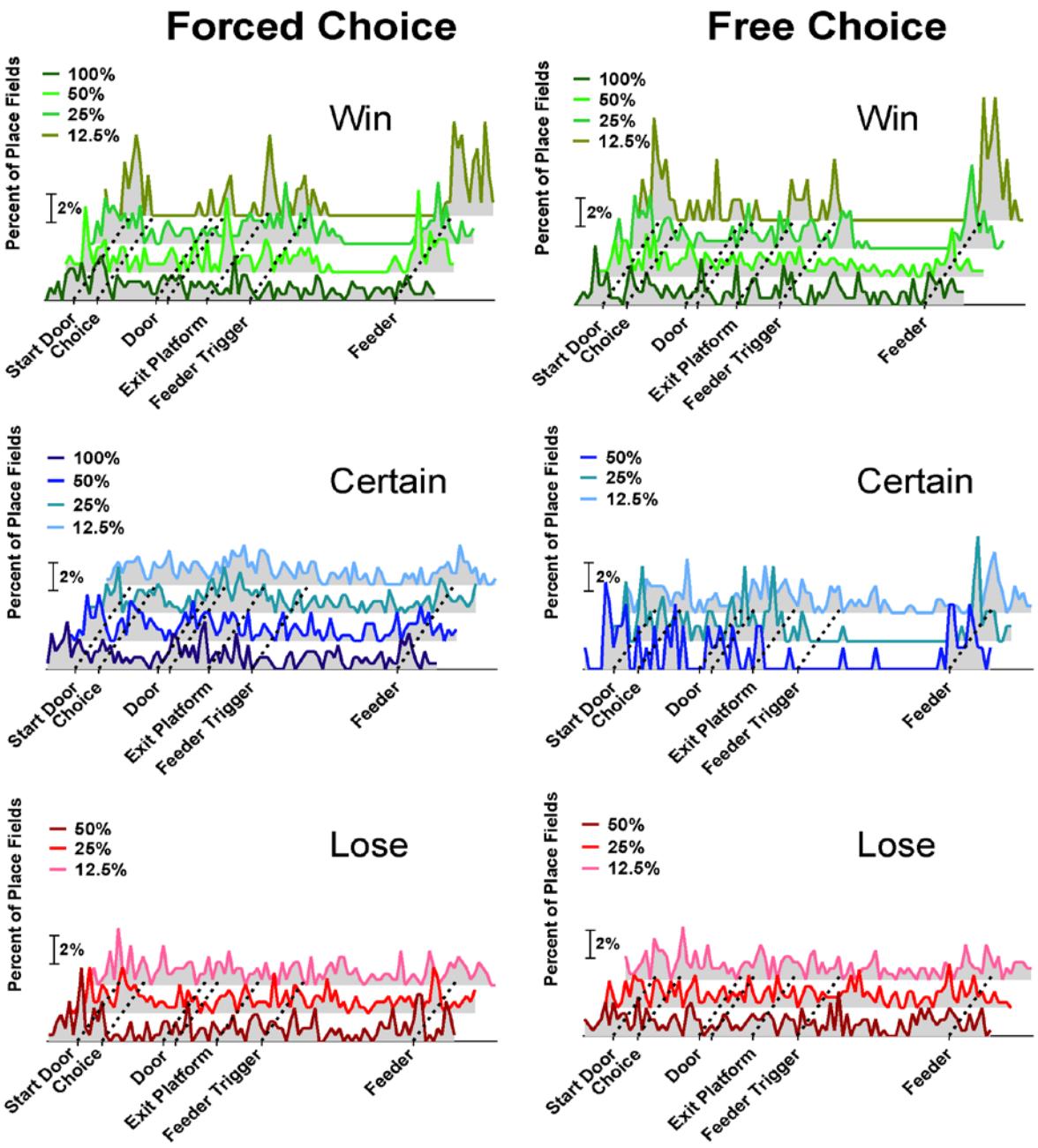

Forced Choice
Free Choice
Win
Certain
Lose
Percent of Place Fields
100%
50%
25%
12.5%
2%
Start Door Choice
Door
Exit Platform
Feeder Trigger
Feeder

**Figure 2.4. The distribution of place fields varied depending on the expected reward probability as shown on linearized representations of the loop maze.** Distributions of place fields during forced choice (left) or free choice (right) trials are shown as a function of outcome (win-top, certain-middle, and lose-bottom). Within each plot, we illustrate the proportion of place fields as a function of probability of reward. During win trials, a larger proportion of place fields were found around encounters with the feeder, and this was most pronounced on low probability trials. This pattern was also observed, albeit to a lesser extent, during free choice, certain trials, but not during force choice, certain or lose trials.

**Tables 1 and 2: Chi square and binomial test results from analyses of place field distributions**. To determine whether or not place fields were distributed in a biased fashion across the maze, the frequencies of place fields in the eight regions of the maze were assessed using the Chi square test. The null hypothesis was that the place fields were evenly distributed across the different regions of the maze, adjusted for the size of each of the eight regions. $p < 0.05$ indicates a non-equal distribution. To specifically test if there were more place fields in the feeder region of the maze, a binomial test was performed comparing the observed and expected number of place fields in the feeder region and the rest of the maze. Expected numbers of place fields were based on the size of the region (i.e. the feeder region was 22% of the maze area so 22% of place fields would be expected there). $p < 0.05$ indicates there were significantly more or less fields in the feeder region than expected.

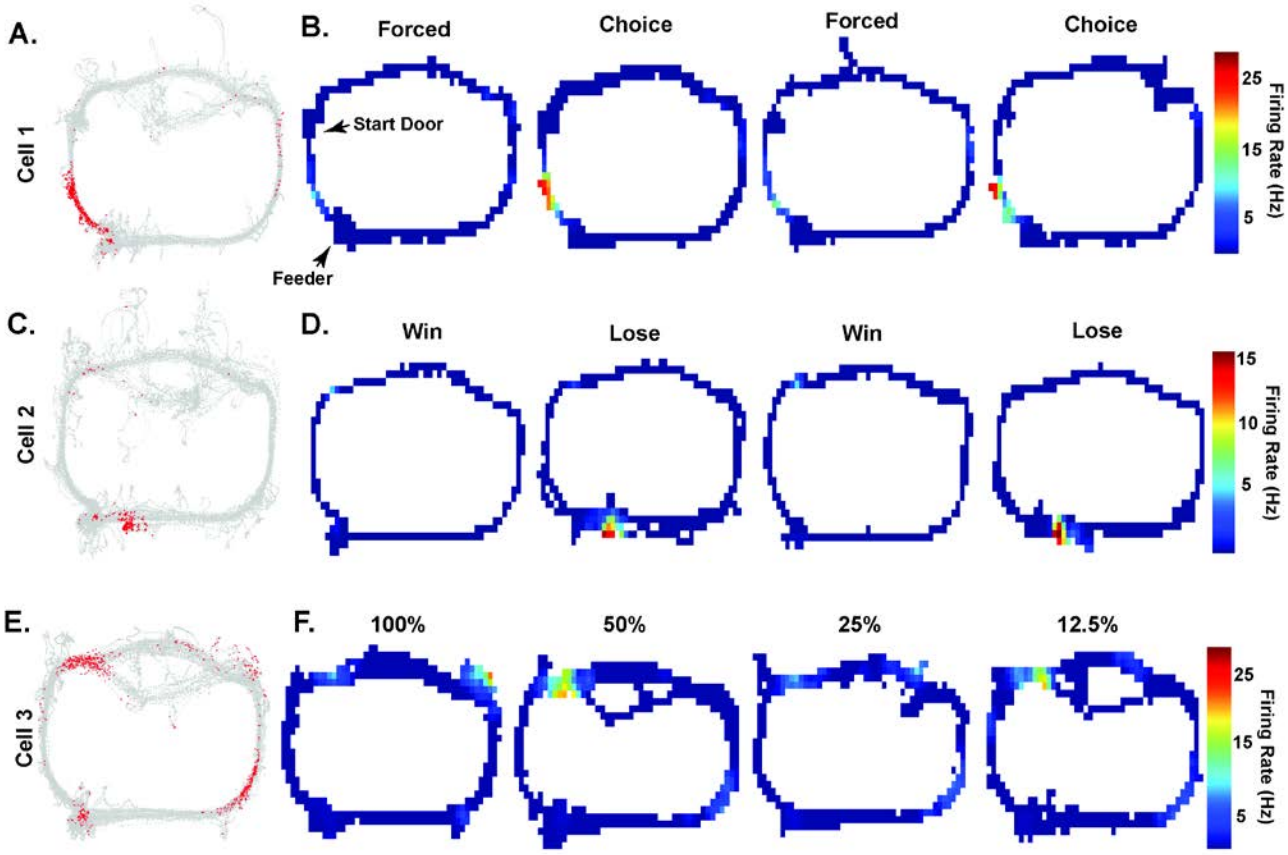
**Table 1**

| Probability | Agency | Outcome | $X^2$ | *p* value |
|---|---|---|---|---|
| 100% | Forced | Win | 21.77 | 0.003 |
| 100% | Choice | Win | 7.91 | 0.335 |
| 50% | Forced | Win | 33.76 | 0.000 |
| 50% | Choice | Win | 16.64 | 0.020 |
| 25% | Forced | Win | 50.33 | 0.000 |
| 25% | Choice | Win | 50.41 | 0.000 |
| 12.5% | Forced | Win | 79.09 | 0.000 |
| 12.5% | Choice | Win | 45.81 | 0.000 |
| 100% | Forced | Certain | 27.54 | 0.000 |
| 50% | Forced | Certain | 22.48 | 0.002 |
| 50% | Choice | Certain | 28.265 | 0.000 |
| 25% | Forced | Certain | 28.947 | 0.000 |
| 25% | Choice | Certain | 49.45 | 0.000 |
| 12.5% | Forced | Certain | 38.38 | 0.000 |
| 12.5% | Choice | Certain | 28.08 | 0.000 |
| 50% | Forced | Lose | 21.51 | 0.003 |
| 50% | Choice | Lose | 11.14 | 0.132 |
| 25% | Forced | Lose | 9.238 | 0.236 |
| 25% | Choice | Lose | 13.29 | 0.065 |
| 12.5% | Forced | Lose | 6.61 | 0.470 |
| 12.5% | Choice | Lose | 17.41 | 0.015 |

**Table 2**

| | Observed | | Expected | | | p value | |
|---|---|---|---|---|---|---|---|
| | **Feeder Region** | **Rest of Maze** | **Feeder Region** | **Rest of Maze** | **Total** | **one-tailed** | **two-tailed** |
| 100% Forced Win | 39 | 184 | 49.06 | 173.94 | 223 | 0.06 | 0.11 |
| 100%Choice Win | 45 | 169 | 47.08 | 166.92 | 214 | 0.40 | 0.80 |
| 50% Forced Win | 44 | 128 | 37.84 | 134.16 | 172 | 0.15 | 0.27 |
| 50% Choice Win | 40 | 192 | 51.04 | 180.96 | 232 | 0.04 | 0.08 |
| 25% Forced Win | 36 | 147 | 40.26 | 142.74 | 183 | 0.25 | 0.48 |
| 25% Choice Win | 44 | 142 | 40.92 | 145.08 | 186 | 0.32 | 0.60 |
| 12.5% Forced Win | 45 | 60 | 23.1 | 81.9 | 105 | <0.0001 | <0.0001 |
| 12.5% Choice Win | 28 | 41 | 15.18 | 53.82 | 69 | 0.0004 | 0.0006 |
| 100% Forced Certain | 43 | 193 | 51.92 | 184.08 | 236 | 0.09 | 0.18 |
| 50% Forced Certain | 48 | 196 | 53.68 | 190.32 | 244 | 0.21 | 0.44 |
| 50% Choice Certain | 18 | 47 | 14.3 | 50.7 | 65 | 0.17 | 0.29 |
| 25% Forced Certain | 47 | 197 | 53.68 | 190.32 | 244 | 0.17 | 0.35 |
| 25% Choice Certain | 21 | 73 | 20.68 | 73.32 | 94 | 0.51 | 0.90 |
| 12.5% Forced Certain | 46 | 202 | 54.56 | 193.44 | 248 | 0.11 | 0.22 |
| 12.5% Choice Certain | 47 | 161 | 45.76 | 162.24 | 208 | 0.44 | 0.80 |
| 50% Forced Lose | 52 | 159 | 46.42 | 164.58 | 211 | 0.20 | 0.36 |
| 50% Choice Lose | 59 | 194 | 55.66 | 197.34 | 253 | 0.33 | 0.60 |
| 25% Forced Lose | 52 | 192 | 53.68 | 190.32 | 244 | 0.43 | 0.88 |
| 25% Choice Lose | 54 | 205 | 56.98 | 202.02 | 259 | 0.36 | 0.71 |
| 12.5% Forced Lose | 49 | 199 | 54.56 | 193.44 | 248 | 0.22 | 0.44 |
| 12.5% Choice Lose | 55 | 187 | 53.24 | 188.76 | 242 | 0.42 | 0.76 |

While analyzed at the population level, the ability of the econometric variables to influence spatially selective firing was observed at the single-field level **Figure 2.5** shows the rats' trajectory in gray and all of the recorded spikes for a cell over the entire session are depicted in red. Figure 3A and B show a hippocampal neuron's clear place field during free choice trials (Choice) relative to forced choice trials (Forced) of the same probability block. This place field did not vary significantly across probability blocks, but rather by agency. Other place cells preferentially fired during trials of a particular outcome rather than agency or probability (**Fig. 2.5C &D**). Also shown is a place field around the location of the reward but only on lose trials. For another hippocampal neuron, it can be seen that different spatial firing patterns of place fields were observed when the probability of reward varied from 100% to 12.5% even though the sensory environment, behavioral responses, and motivation were the same (**Fig. 2.5E, F)**. It can be seen that little firing was observed for the 100% and 25% blocks, and the highest firing rates were seen for the 50% and 12.5% blocks of trials. Forced and free choice trials were collapsed in this example as no differences were observed.




**<u>Figure 2.5</u>. Individual examples of place field responses to the expected probability of receiving a large reward, agency, and trial outcome.** Plots shown in **A, C, and E** show the rats' trajectory in gray and all of the recorded spikes for a cell over the entire session is depicted in red. Heat maps in **B, D, and F** depict the spatial firing patterns during different conditions. **A, B:** A hippocampal neuron shows clear place field activity during free choice trials (Choice) relative to forced choice trials (Forced) of the same probability block. This place field did not vary significantly across probability blocks, but rather by agency. **C, D:** Other place cells preferentially fired during trials of a particular outcome rather than agency or probability. Shown is a place field that was observed around the location of the reward but only on lose trials. **E, F:** For another hippocampal neuron, it can be seen that different spatial firing patterns of place fields were observed when the probability of reward varied from 100% to 12.5% even though the sensory environment, behavioral responses, and motivation were the same. It can be seen that little firing is observed for the 100% and 25% blocks, and the highest firing rates were seen for

the 50% and 12.5% blocks of trials. Forced and free choice trials were collapsed in this example as no differences were observed.

Place fields change primary field location and mean firing rate as a function of expected probability of reward, agency and choice outcome: A multiple regression was run to predict the location of the primary place field from agency, probability, and outcome **(Fig. 2.6)**. First, neurons were not included in further analyses if the mean rate over the entire recording session was not at least 0.1 Hz or greater than 10 Hz. A total of 364 neurons met this initial rate criterion. The three econometric variables significantly predicted the location the of the primary place field for 166 neurons, ($p \leq .05$, mean $R^2 = .24$). Only one cell had all three variables as significant predictor of location whereas 40 cells had 2 significant predictors of location. Of the 40 cells with two significant predictors of location, 10 were agency and outcome, 10 were agency and probability, and 20 were probability and outcome. 125 cells had only one significant predictor; $p \leq .05$.These variables did not significantly predict location of the primary place field for 197 neurons (54%).

Another multiple regression was run on the same 364 neurons to predict the rate of the primary place field as a function of agency, probability, and outcome. These variables statistically significantly predicted the mean firing rate of the primary place field for 168 (or 46% of) neurons, ($p \leq .05$, mean $R^2 = .23$). Only two cells had all three variables as significant predictor of rate whereas 44 cells had two significant predictors of rate. Of the 44 cells with two significant predictors of rate, 8 were agency and outcome, 10 were agency and probability, and 26 were probability and outcome. 124 cells had only one significant predictor; $p \leq .05$.These variables did not significantly predict the rate of the primary place field for 195 neurons (54%).

If one of the variables was a significant predictor of rate or location in the multiple regression, it is represented in **Figure 2.6** where the mean beta (unstandardized) coefficient value represents the degree to which significant predictors influence either rate or location. At a population level,

outcome had the strongest impact on place fields location followed by probability (which matches the place field distribution data described below). Of those place fields whose locations were affected by econometric factors, the mean unstandardized coefficient shows that agency and outcome had the biggest impact, causing the largest shift in location. When looking at rate, probability and outcome had similar impacts. Mean unstandardized coefficient weight data show that of the impacted cells, agency caused the largest change in firing rate, regardless of location.

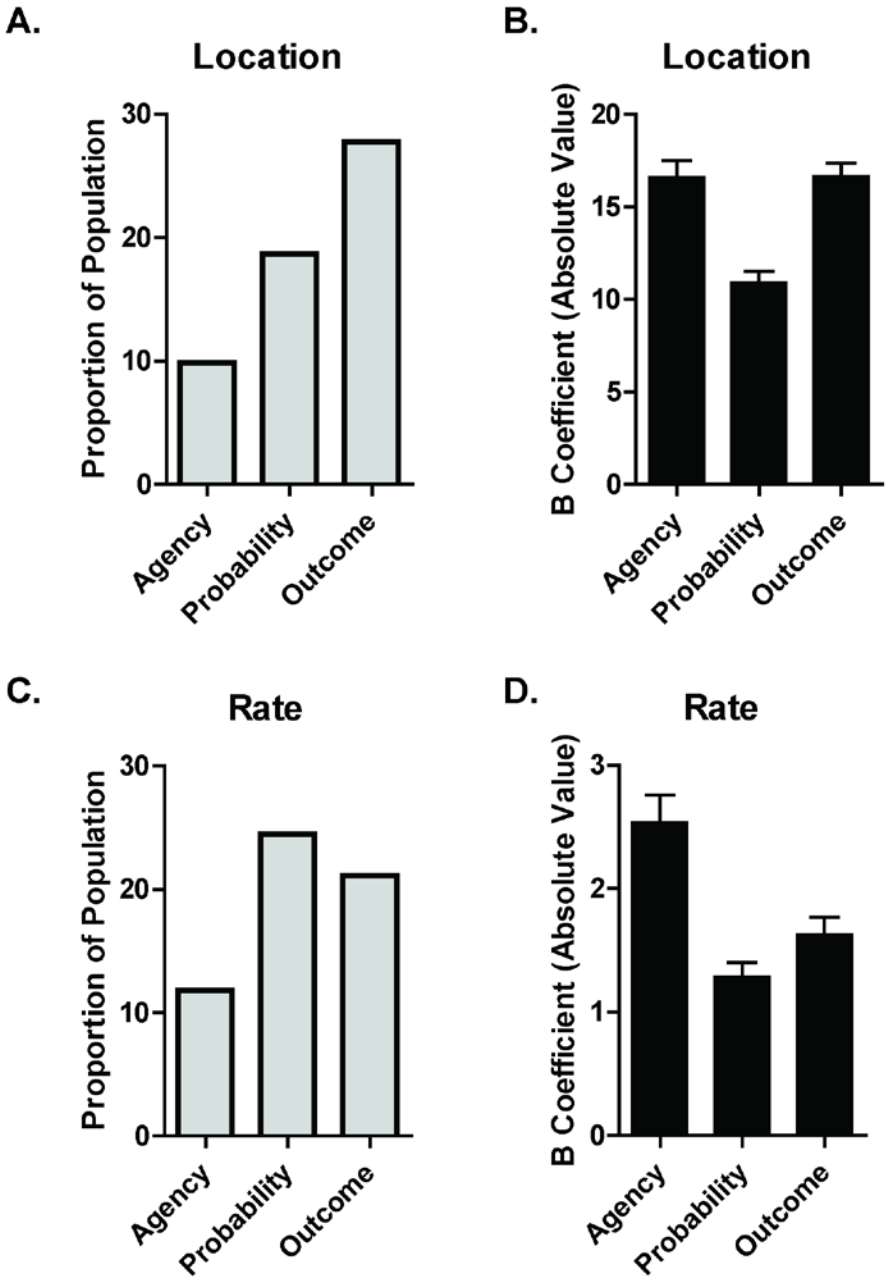



**Figure 2.6. Place fields change primary field location and mean firing rate as a function of expected probability of reward, agency and choice outcome:** A multiple linear regression was performed on 364 neurons to determine if agency, probability, or outcome could significantly predict changes in primary place field location and/or rate. **A.** Econometric variables significantly predicted the location the of the primary place field for 166 neurons, ( $p \leq .05$, mean $R^2 = .24$). The y axis represents the proportion of the tested neurons for which a single econometric variable was a significant predictor of location of the primary place field. Agency was a significant predictor of location for 9.89% of neurons, probability for 18.68%,

and outcome for 27.75% of the neurons tested. **B.** The mean B coefficient values (y axis) are the degree to which significant predictors influence location of the primary place field. A whole integer represents the bin location on the linearized maze. **C.** Econometric variables significantly predicted the mean firing rate of the primary place field for 168 neurons, ( $p \leq .05$, mean $R^2 = .23$). The y axis represents the proportion of the tested neurons for which a single econometric variable was a significant predictor of mean firing rate of the primary place field. Agency was a significant predictor of rate for 11.81% of neurons, probability for 24.45%, and outcome for 21.15% of the neurons tested. **D.** The mean B coefficient values (y axis) are the degree to which significant predictors influence the mean firing rate of the primary place field. $\alpha$ was set at $p \leq 0.05$. Error bars represent the ±SEM.

Hippocampal theta oscillations encode econometric information: LFP data were continuously recorded from the same tetrodes that recorded single unit data. Described below are theta band (6-10 Hz) power changes in response to different economic conditions of choice.

*Theta power correlates with economic aspects of choice options*: **Figure 2.7** illustrates changes in theta power as rats traversed (a linearized representation of) the maze. Also shown (black dashed line) is the average velocity with which rats moved across the maze. **Figure 2.7A** separates theta power as a function of choice outcome (win, lose, certain).

To illustrate the impact of probability and agency conditions on the differential theta response to choice outcome, **Figure 2.7B** presents theta power as a function of outcome (win trials-top row, certain trials-middle row, and lose trials-bottom row) for forced choice trials (left column) and free choice trials (right column).

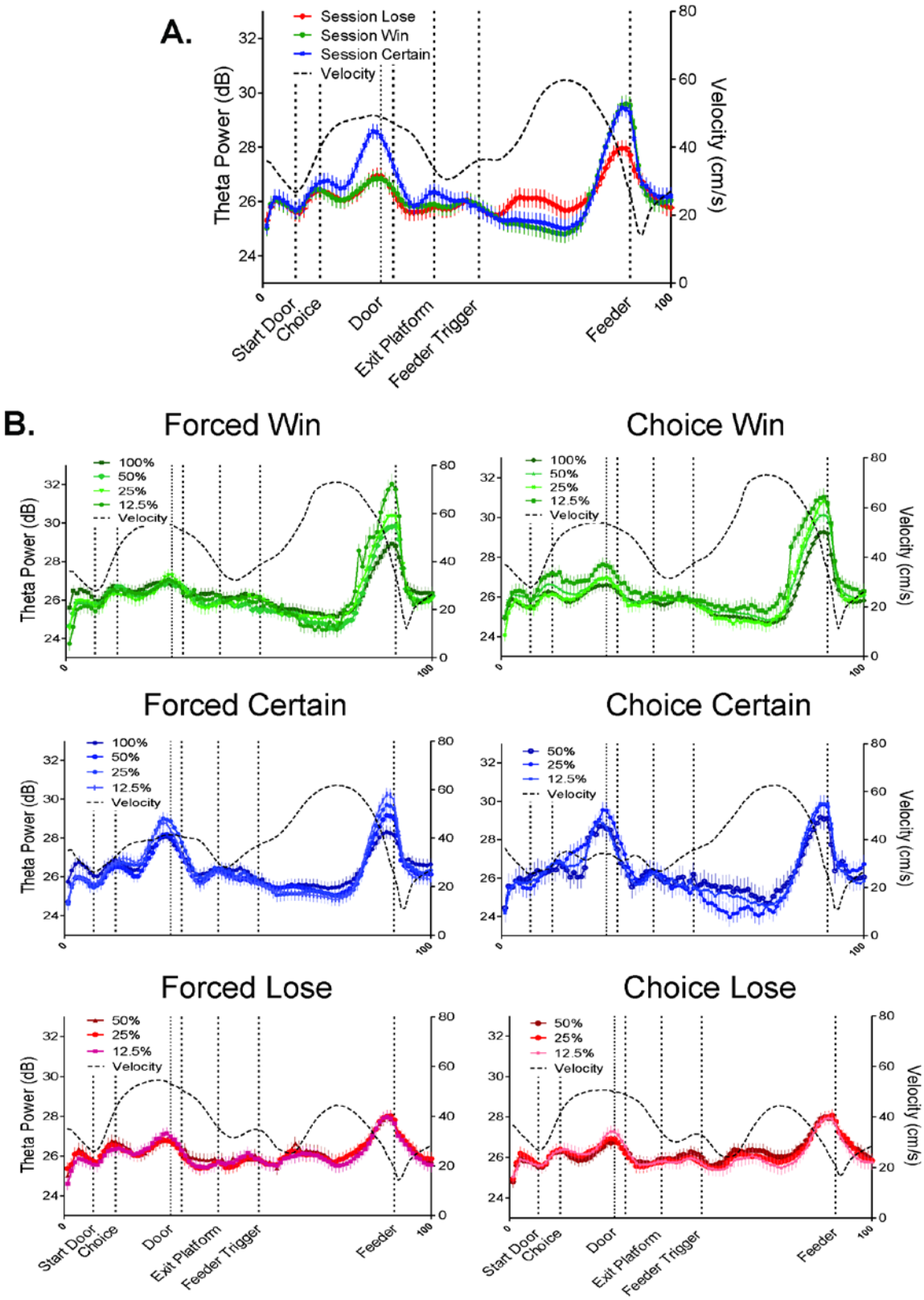

A.
Session Lose
Session Win
Session Certain
Velocity
Theta Power (dB)
Velocity (cm/s)
Start Door
Choice
Door
Exit Platform
Feeder Trigger
Feeder
B.
Forced Win
Choice Win
Forced Certain
Choice Certain
Forced Lose
Choice Lose
100%
50%
25%
12.5%
Velocity
Theta Power (dB)
Velocity (cm/s)

**Figure 2.7. Power of the theta rhythm varied according to changing economic conditions of choice. A.** Mean (±SEM) theta power is compared across choice outcomes on a linearized representation of the loop maze. While some variability is observed, particularly striking is the peak in theta power that starts prior to reward (feeder) encounters. Most notable, the greatest theta response is observed around the feeder, particularly for win and certain trials. The mean velocity of the rat is shown by the black dashed line. It can be seen that the overall fluctuations in theta power did not track directly variations in velocity, suggesting that other factors contributed to the theta changes. **B.** The whole session data shown in A) are broken down according to agency (forced choice-left column; free choice-right column) and choice outcome (win, certain, lose) within each panel. Theta data are further broken down according to the expected probability of reward. Several patterns of results are clear. The increased theta power around the feeder is not only greatest for win trials, followed by certain trials (and very little for lose trials), but the magnitude of the increased theta power scales to the expected probability of reward. That is, the smallest increase in theta was observed during 100% win trials (after both forced and free choices), and the largest increase in theta power was observed during 12.5% win trials. A similar type of scaling was observed during the forced choice, certain trials.

The changes in theta power observed (**Fig. 2.7**) suggest that theta is related to behavior in different ways during task performance. The loop maze was designed so that different segments of the maze were associated with the presentation/processing of specific types of information and opportunities (e.g., choices) to the rat. In this way, it was possible to compare the relative contribution of not only probability, agency, choice outcome, and velocity to behavioral and neural responses, but also to demonstrate the contributions of meaningful and specific information gleaned by rats as they traversed the maze. **Figure 2.9A** illustrates and defines these different informational regions of the maze. For each maze segment, theta power for each tetrode during each trial was determined and a multiple linear regression analysis was run for each region with agency, outcome, probability, and velocity as predictors. As predicted from numerous past studies (e.g., Whishaw and Vanderwolf, 1973; Buzsaki 2002), velocity was a significant predictor of theta power fluctuation in all segments (**Fig 2.9B).** Therefore, only the econometric variables that influenced theta power will be discussed below.

While the rat resided in the pre-decision area of the maze (*region 1*), probability and agency were significant predictors of theta power while outcome was not (model $F$ (4, 38919) = 665.18, $p < 0.001$, unstandardized coefficient for agency: 0.07, $p = .02$, probability: 0.11, $p < 0.001$, outcome: 0.02, $p = .68$, and velocity: 0.16, $p < 0.001$). This might have been expected as agency and probability information (based on the previous trial) were known to the rat in this region. For the decision platform, (*region 2*) agency information should be salient to the animal because they are either forced to make a particular decision or are able to freely choose between the two options. Consistent with this view, we found that only agency could explain a significant amount of the variability observed in theta power in region 2 (model $F$ (4, 38243) = 2161.08, $p <$

0.001, unstandardized coefficient for agency: 0.11, $p = 0.01$, probability: 0.01, $p = 0.71$, outcome: 0.05, $p = .06$, and velocity: 0.08, $p < 0.001$).

The immediate post decision region of the maze was demarcated by the sensor that was triggered when the animal passed through the decision doors but ended before the rat knew the outcome of its choice. While rats were on this maze segment (*region 3*), they may have retained information about the nature of the choices made, resulting in continued modulation of theta power by agency. In addition, probability information again significantly modulated theta power, while outcome did not, presumably because the outcome was not yet known for risky choices (model $F(4, 38253) = 291.50$, $p < 0.001$, unstandardized coefficient for agency: 0.23, $p < 0.001$, probability: 0.28, $p < 0.001$, outcome: 0.06, $p = 0.14$, and velocity: 0.11, $p < 0.001$). The next segment (*region 4*) was defined as the region after exiting the decision platform, a time when the rat heads toward the feeder trigger. All three econometric variables significantly influenced theta power in this region even though the rat did not yet know the outcome of risky choices (model $F(4,38243) = 469.65$, $p < 0.001$, unstandardized coefficient for agency: 0.12, $p = 0.03$, probability: 0.25, $p < 0.001$, outcome: 0.36, $p < 0.001$, and velocity: 0.24, $p < 0.001$). It is possible that expected or predicted outcome of a choice influenced the rats' evaluation of the decision.

New information was provided in *region 5* as it contained the feeder trigger sensor which provided the rat with information regarding the outcome of its most recent choice, as well as information about the magnitude of the expected reward (i.e one click was audible per pellet dispensed). Therefore, it was hypothesized that if theta is related to specific learned goal information, outcome should now account for a disproportionately greater amount of the variance in theta power. Indeed, we observed a large increase in the variability in theta power

that could be explained by outcome. Agency was still related to theta power in this region while probability was not (model $F$ (4, 38243) = 104.67, $p < 0.001$, unstandardized coefficient for agency: 0.19, $p < 0.001$, probability: 0.01, $p = 0.64$, outcome: 1.24, $p < 0.001$, and velocity: 0.08, $p < 0.001$).

The post-feeder trigger region (*region 6*) was the maze area between the time when animals received the outcome information for that trial, and the time when the rat arrived at the food cup. In this region, outcome continued to have a significant effect on theta power, as did probability. Agency, however, had little or no impact on theta power at this point of maze performance (model $F$ (4, 38243) = 61.39, $p < 0.001$, unstandardized coefficient for agency: 0.04, $p = 0.55$, probability: 0.13, $p < 0.001$, outcome: 0.48, $p < 0.001$, and velocity: 0.01, $p = 0.01$). This could be due, at least in part, to the fact that the ability to make a choice is irrelevant at this point in the task.

The rat arrived at the reward location during the next segment of the maze (*region 7*). The end of this region is demarcated by the sensor at the feeder. Theta power was dramatically elevated just prior to reward encounters, on average regardless of whether the reward was large (win condition) or small (certain condition). In contrast, a smaller increase in theta was observed at the reward location on lose trials. Such a distinguished response was also evident in single tetrode records (**Fig. 2.8**). This is the first identification of a specific type of economic information (and not just ongoing behavior) encoded by theta. In alignment with this qualitative observation that reward and goal information significantly shapes theta, outcome again predicted a large amount of variability in theta power in this region (model $F$ (4, 38243) = 331.32, $p < 0.001$, unstandardized coefficient for agency: 0.06, $p = 0.24$, probability: 0.22, $p < 0.001$, outcome: 1.42, $p < 0.001$, and velocity: 0.11, $p < 0.001$). During rewarded trials, it appears that

the increase in theta power prior to reward encounters scaled inversely with the probability condition of the trial. That is, theta power is greatest during the lowest probability condition. The elevated theta could not be due to the presence of reward per se since the rats received the same type and magnitude of rewards for all win trials (regardless of probability condition), or due to the specific behaviors of the rats (since in all win trials, rats expected rewards at the feeder and stopped to consume the food). Rather theta appeared to respond to the known probability condition of a block. Probability scaling of theta was observed not only in the population summary (**Fig. 2.7**) but also for individual tetrode recording sites (**Fig. 2.8B**). Thus, not surprisingly, probability was also significant predictor of theta power in this region, which could explain the interaction between probability of receiving reward and the magnitude of reward being reflected in the scaling of theta power at the reward location (**Fig 2.7B**.). Finally, the last region of the maze (*region 8*), includes the maze area just after the food encounter. Here, we found that only agency and outcome had a modest influence on the variability observed in theta power (model $F$ (4, 38010) = 7.96, $p < 0.001$, unstandardized coefficient for agency: 0.13, $p = 0.03$, probability: 0.05, $p = 0.13$, outcome: 0.09, $p = 0.03$, and velocity: 0.02, $p < 0.001$).

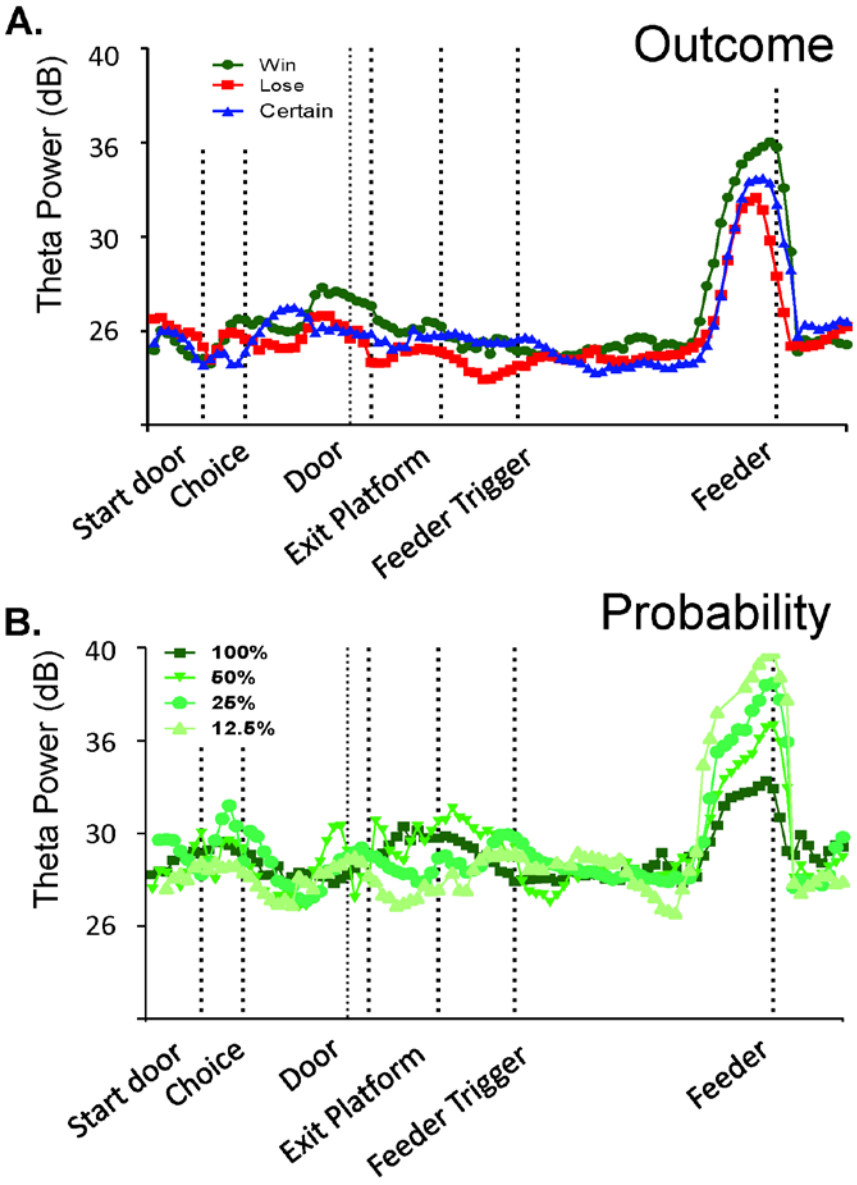
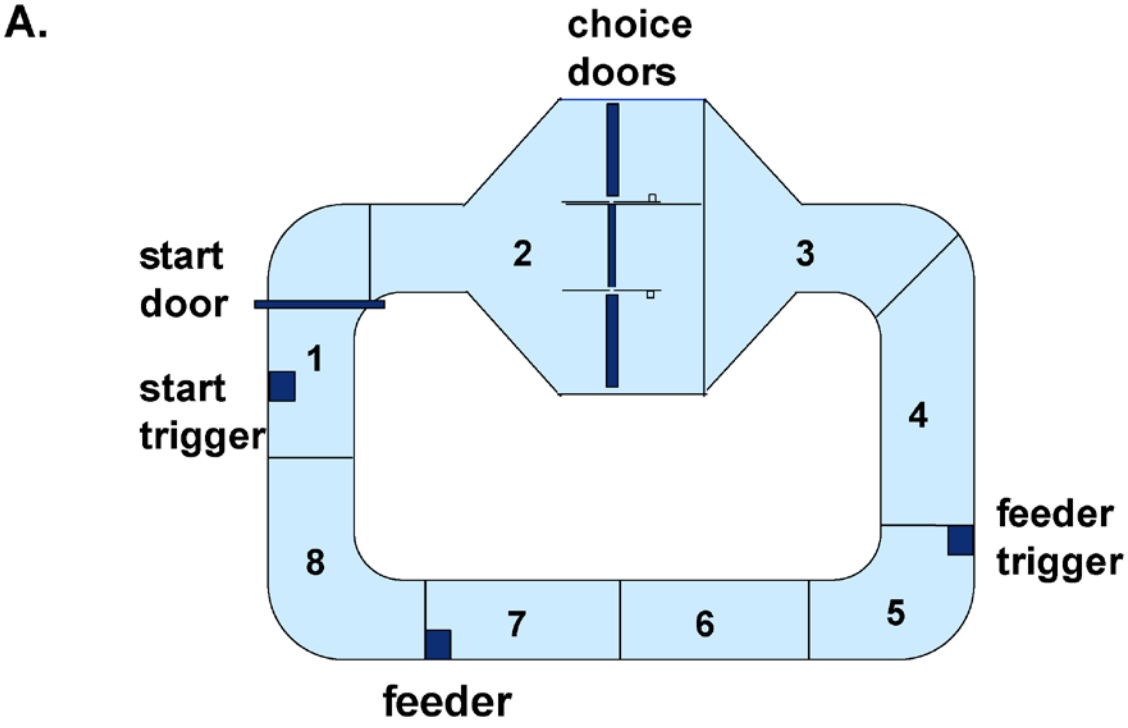

A.
Outcome
Win
Lose
Certain
Theta Power (dB)
40
36
30
26
Start door
Choice
Door
Exit Platform
Feeder Trigger
Feeder
B.
Probability
100%
50%
25%
12.5%
Theta Power (dB)
40
36
30
26
Start door
Choice
Door
Exit Platform
Feeder Trigger
Feeder

**Figure 2.8. Theta power modulation according to the expected reward outcome and probability is observed on single tetrode traces. A.** Theta power was dramatically elevated just prior to reward encounters at the feeder, with the increase greatest during win trials, followed by certain trials. Lose trials showed the smallest increase. **B**. Single tetrode example of the inverse scaling of the increase in theta power as a function of the expected probability of reward during win trials. These data were collected during a single recording session. The fact that the scaled response can be seen in a single recording site illustrates that the group summary shown in Figure 5 is not to the averaging across a large number of sessions.


A.
choice
doors
start
door
start
trigger
1
2
3
4
5
6
7
8
feeder
trigger
feeder

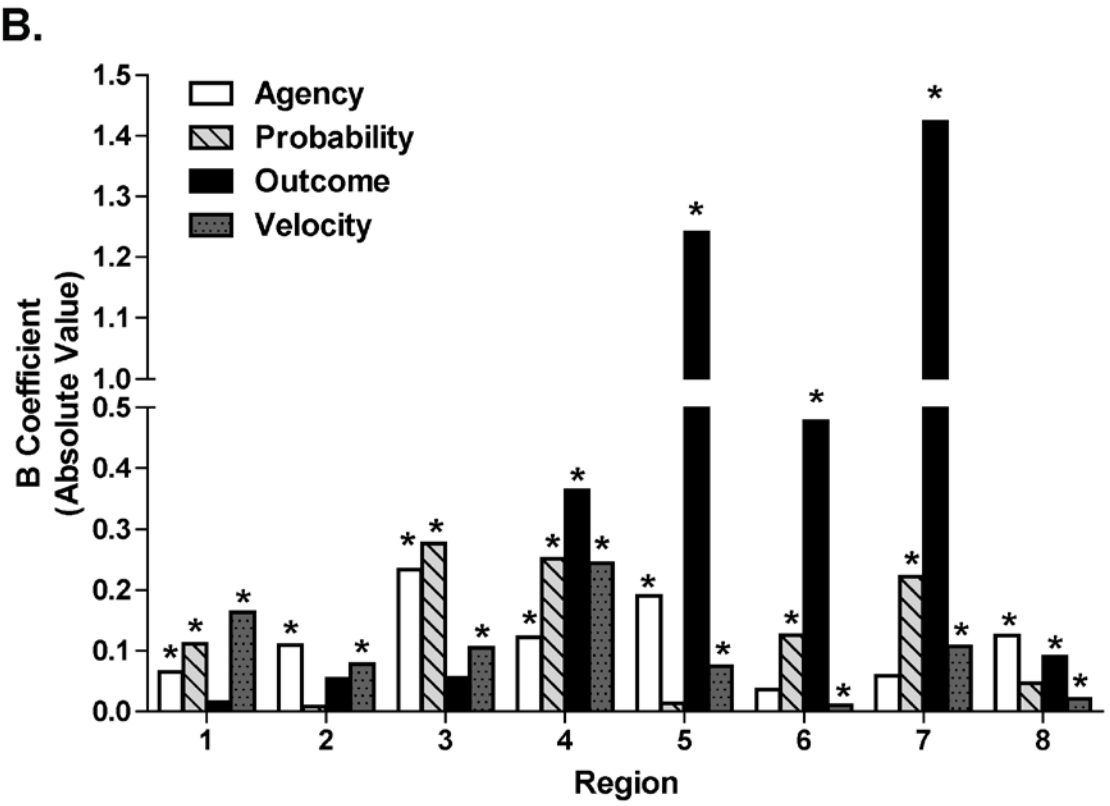

B.
Agency
Probability
Outcome
Velocity
B Coefficient
(Absolute Value)
1.5
1.4
1.3
1.2
1.1
1.0
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
8
Region

**Figure 2.9. The impact of reward probability, agency and expected choice outcome on theta power varies depending upon the economic information available at different regions of the loop maze. A**. The maze was divided into eight regions that were associated with varying types of information processing (see text). **B**. For each maze region, the amount of variability in theta power (for each tetrode during each trial) that could be accounted for by probability, agency and choice outcome was determined with a multiple linear regression analysis (* $p < .05$), using agency, probability, outcome and velocity as predictors. Velocity significantly modulated theta across the entire maze. In contrast, theta was differentially modulated depending on the region of the maze the rat was in, and thus the information that was available at particular task phases. Importantly, agency and probability tended to have the strongest influence until the rat received the reward predicting cue. After the cue presentation, outcome was significantly related to theta power. Together, these data illustrate that theta power is modulated according different types of economic information that is available during goal-directed navigation.

Since theta power increases around the goal appeared similar to that observed for place fields, we were interested to see if dynamic changes in theta power were related to place field characteristics, such as changes in in-field firing rate and location of primary place fields. To assess this, a Pearson's correlation analysis was performed to compare mean theta power over the session for each linearized bin of the maze with the in-field firing rate of a primary place field that occurred in the same linear bin. We also tested whether mean theta power over the session for each linearized bin correlated with the frequency of place fields that occurred in that corresponding bin. Theta power was not correlated with in-field firing rate of primary place fields ($r^2 = 0.02$, $p = 0.15$) but was significantly correlated with the frequency at which place fields occurred in a particular location ($r^2 = 0.12$, $p = 0.05$) (**Fig. 2.10A & B**). From these data, it is apparent that instantaneous theta power has a significant relationship with the number of primary place fields that occurred at particular locations rather than the mean rate of those fields (**Fig. 2.10)**.

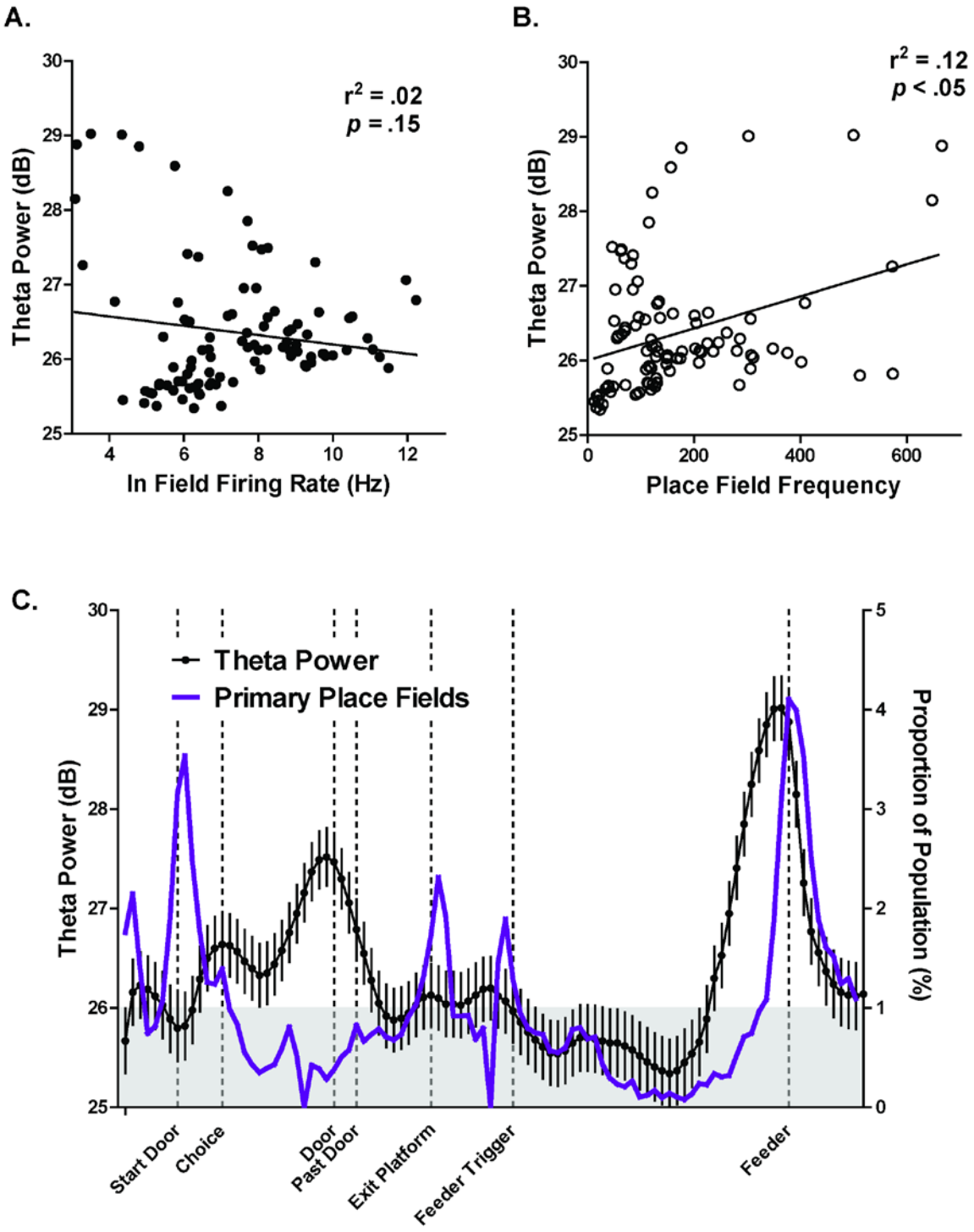
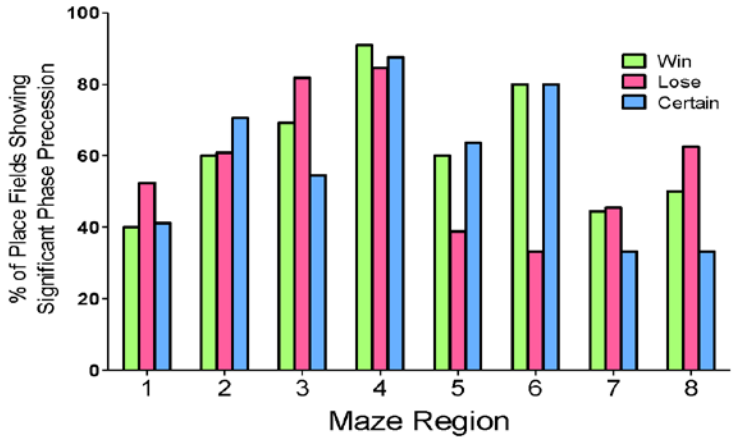

A.
$r^2$ = .02
p = .15
Theta Power (dB)
In Field Firing Rate (Hz)
B.
$r^2$ = .12
p < .05
Theta Power (dB)
Place Field Frequency
C.
Theta Power
Primary Place Fields
Theta Power (dB)
Proportion of Population (%)
Start Door
Choice
Door
Past Door
Exit Platform
Feeder Trigger
Feeder

**Figure 2.10. Instantaneous theta power is significantly related to the number of primary place fields that occur at particular locations but not the mean rate of those fields. A.** Pearson's correlation between mean theta power (y axis) over the session for each linearized bin of the maze and the in-field firing rate of a primary place field (x axis) that occurred in the same linear bin revealed no significant relationship between these two variables. **B.** Pearson's correlation between mean theta power (y axis) over the session for each linearized bin and the frequency of place fields (x axis) that occurred in that corresponding bin. This analysis revealed that theta power and place field frequency are significantly correlated. **C.** Linearized representation of mean theta power (left y axis) over the loop maze plotted with frequency of primary place fields (right y axis). The shaded gray region corresponds to chance levels of place field occurrence. Error bars on theta power represent ±SEM.

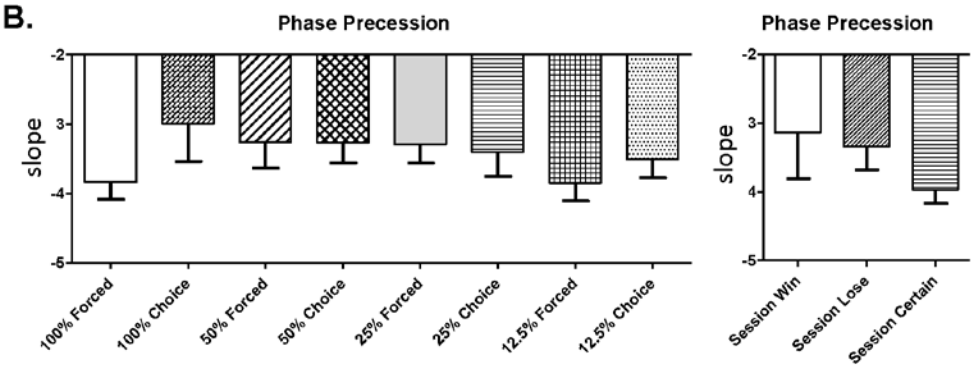
Overall, econometric variables clearly and differentially influenced the strength of theta oscillations depending on the area of maze that the rat was experiencing, and thus what information was available and useful to the rat during task performance. This was evident from the finding that the outcome variable predicted the greatest amount of fluctuation in theta power after the outcome was known, supporting the hypothesis that theta is significantly influenced by goal and reward information.

*Theta phase precession does not reflect economic information*: No differences in theta phase precession were observed as a function of the expected probability of reward, agency, choice outcome. However, the percent of place fields showing significant phase precession did vary by segment of the maze (2-way ANOVA, main effect of region, $F(2,7) = 3.497$, $p = 0.02$. No effect of outcome $F(2,7) = 0.225$, $p = 0.80$) (**Fig. 2.11**). It is noteworthy that the proportion of place fields showing phase precession steadily increased until rats were given information about the outcome of their last choice. This suggests that the association between place fields and theta precession is related to information processing that remains engaged until the outcomes of choices are known.

A.


% of Place Fields Showing Significant Phase Precession
100
80
60
40
20
0
Win
Lose
Certain
1
2
3
4
5
6
7
8
Maze Region

B.


Phase Precession
slope
-2
-3
-4
-5
100% Forced
100% Choice
50% Forced
50% Choice
25% Forced
25% Choice
12.5% Forced
12.5% Choice
Phase Precession
slope
-2
-3
-4
-5
Session Win
Session Lose
Session Certain

C.

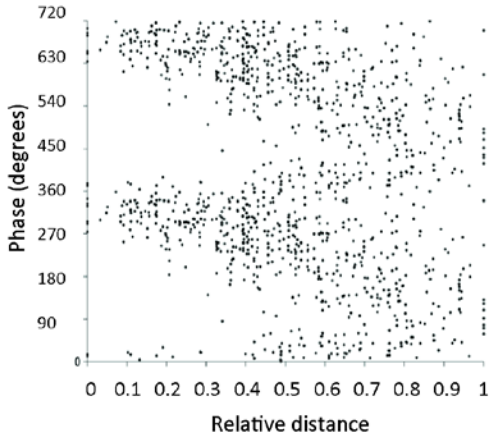

Phase (degrees)
720
630
540
450
360
270
180
90
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Relative distance

**Figure 2.11. Degree of phase precession is not significantly modulated by probability, agency or outcome. A.** The percent of place field passes that showed significant phase precession as a function of reward probability, agency, and choice outcome is shown as a function of maze region (regions are defined in Fig. 7) (2-way ANOVA, main effect of region, $F(2,7) = 3.497$, $p = 0.02$. No effect of outcome $F(2,7) = 0.225$, $p = 0.80$). **B**. The average (mean $\pm$ SE) slope of the theta phase relationship relative to place cell firing. No statistical differences were observed in terms of the degree of phase precession and probability of reward, agency, or choice outcome. **C**. An illustration of theta precession recorded across an entire recording session.

Ventral tegmental area (VTA) dopaminergic neurons responded to the auditory cues that predicted a rewarded outcome: Expected choice outcome, especially during risky trials, significantly influenced the distribution of place fields as well as dynamic changes in theta power (**Figs. 2.7 and 2.9**). Specifically, the reward location was disproportionately represented as significantly more place fields were found there. Yet neither place fields nor theta power appeared to respond directly to the reward-predicting auditory cue that signaled a specific outcome. However, it si well known that midbrain dopamine cells respond to cues that predict upcoming rewards (e.g., Schultz et al., 1997). Of particular relevance, then, dorsal hippocampal neurons have dopamine receptors (Ishikawa et al., 1982), the VTA projects to dorsal hippocampus (Gasbarri et al., 1994) and dopamine release in hippocampus may modulate spatially specific firing since temporary inactivation of the VTA disrupts the stability of CA1/CA2 place fields in dorsal hippocampus (Martig & Mizumori, 2010) and dopamine receptor antagonists reduce place field stability (Kentros et al., 2004). Thus, we were interested to see if ventral tegmental area dopamine neurons responded to rewards and reward predicting cues on this task, and whether responses to large risky rewards would be larger for lower probabilities. If true, dopamine signaling from the VTA could provide a possible mechanism for the observed response of place fields and theta to expected outcomes. Additional rats were trained on the same probability discounting maze task as that used for the hippocampal recordings to test dopamine cell responses to the reward predicting cues. VTA dopamine neural activity was verified histologically (**Fig. 2.12A**) via published criteria for identifying dopamine cell signals (Roesch et al., 2007; Jo et al., 2013). Of a total of 61 recorded VTA cells, 36 were considered dopaminergic (**Fig. 2.12B**). These cells showed clear elevated responding to each auditory cue presented during win trials (4 in the case of large reward, 1 for the case of small

(certain) rewards; **Fig. 2.12C-E**). No responses were observed when no cue was presented (lose condition). The dopamine neuron response was most strongly elicited by cues that signaled future rewards and they did not respond to reward consumption itself (**Fig. 2.13**). This effect was apparent at the single neuron level (**Fig. 2.12D**) as well as the population level (**Fig. 2.12C**). To quantify dopamine neuron responses to the feeder trigger cue, we analyzed the firing rate of the dopamine neurons during the first 500 ms after the feeder trigger timestamp. Indeed, outcome significantly influenced the firing of dopamine neurons after the feeder trigger cue (repeated measures ANOVA, significant main effect of outcome: $F$ (2, 35) = 12.41, $p < 0.0001$). Additionally, the response of these neurons to the cue during win trials varied depending on the probability of receiving the reward. As expected based on previous literature (Fiorillo et al., 2003), dopamine neurons displayed greater excitation in response to the cue when the probability of receiving the reward was lower (repeated measures ANOVA, significant main effect of probability: $F$ (3, 35) = 2.774, $p < 0.05$; **Fig. 2.12E**). These data indicate that our observation of place fields aggregating around goal locations according to the expected probability of rewards may be due to upstream neural signaling from VTA dopamine neurons.

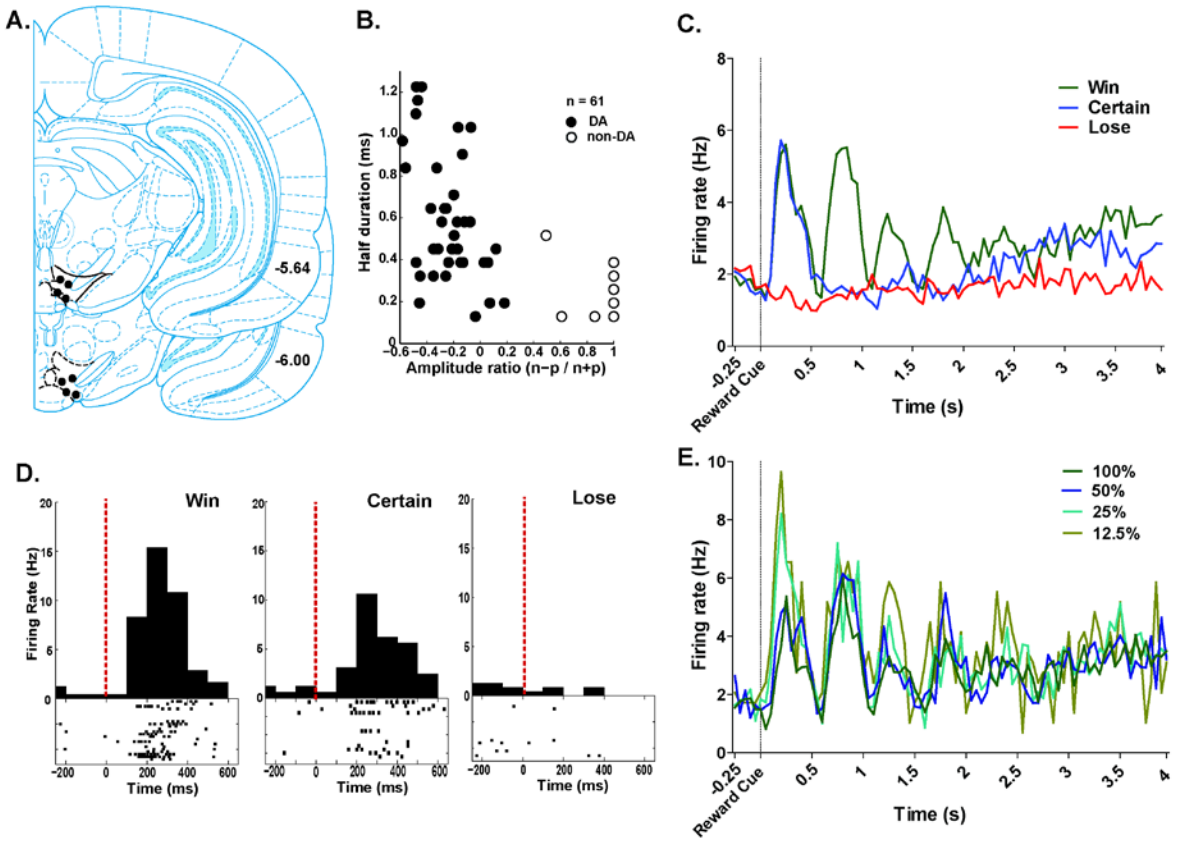



**Figure 2.12. Putative VTA DA neurons respond to the feeder trigger cue that predicts reward: A.** Reconstruction of terminal recording locations for VTA-targeted tetrodes (VTA is outlined in black). **B.** Results of cluster analysis for all VTA neurons (n = 61). Putative dopamine (DA) cells were identified based on half spike duration and the amplitude ratio of the initial negative valley (n) and positive peak (p). **C.** Putative DA neuron responses (n = 36 cells) to the feeder trigger signaling win, certain, and lose trials. [Note: The slight (~100 ms) delay between the time stamp (time 0) and the auditory cue of the motor delivering sugar pellets can account for the apparent delay in neural responding to the cue.] Four peaks are observed for win trials (associated with each of the four feeder triggers that resulted in the delivery of four rewards), one peak was observed for certain trials (associated with the single cue presented for the delivery of one pellet), and no response was observed when no cue was presented on lose trials. **D.** An example DA neuron response to win, certain, and lose feeder trigger cues. The red line indicates the timestamp when the rat tripped the feeder trigger sensor. The greatest initial response was observed during win trials and a smaller response was seen on certain trials. No response was seen on lose trials. **E.** Population summary of responses to the feeder trigger cue on 100%, 50%, 25% and 12.5% win trials. The reward cue indicates the time stamp in the event record when the animal tripped the feeder trigger sensor. It appears that the magnitude of dopamine cell response is greatest when (the same) rewards and cues are given on low probability trials.

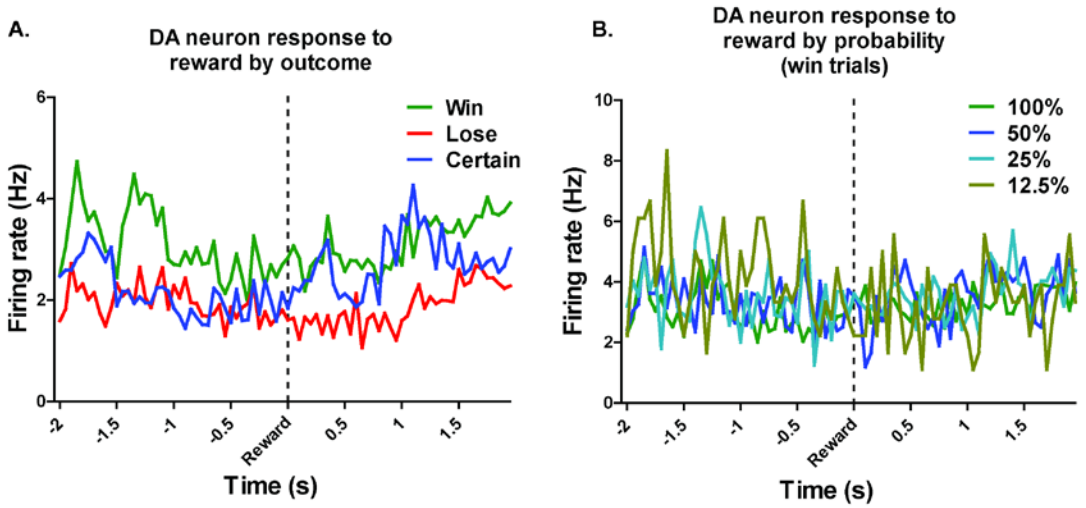



**Figure 2.13. Dopamine neurons did not respond to primary rewards.** To assess if dopamine neuron firing rate was significantly influenced by reward encounters the firing rate of the dopamine neurons during the first 500ms after arrival at the feeder was analyzed. **A.** Outcome significantly influenced the overall firing rate of dopamine neurons (during the 4 second period of the histogram; repeated measures ANOVA, significant main effect of outcome: $F$ (2, 35) = 3.998, $p$ = 0.02). However there was no discrete neural response to reward encounters. **B.** Also, these neurons' firing rate did not vary as a function of the probability of receiving the reward (repeated measures ANOVA, $F$ (3, 19) = 0.112, $p$ = 0.95).

## DISCUSSION

Many past studies clearly show that knowledge about the location of goals impacts decisions and hippocampal neural processing during adaptive navigation. The present study tested directly whether economic considerations of a desired goal also bias behavioral decisions and neural processing by recording place cells and LFPs as rats ran a maze-based probability discounting task. Place fields aggregated near the reward location after the rat received a signal that reward was imminent (i.e. on win trials), and the strength of this biased distribution was most pronounced on low reward probability (win) trials. Since all win trials resulted in the same amount of reward, the probability effect cannot be due to the mere expectation or consumption of a reward. Place fields also distinguished forced and free choice trials in cases when the probability, magnitude, and location of rewards were the same. Theta power (commonly considered to exert a movement-related modulatory effect on place cell excitability, or to be involved in temporally sequencing place fields) was shown to dynamically and differentially vary as a function of expected reward probability, agency, and choice outcome depending on the information that was available to the rat as it traversed different maze regions. Specifically, the theta response scaled to the trial-specific *learned probability* of reward rather than movement or reward acquisition per se. Thus, in addition to goal location, economic factors such as the likelihood of finding reward at a particular location regulates patterned neural activity in hippocampus.

### Place fields show selective encoding of learned probability, agency and choice outcome information

Many reports of hippocampal place field responses to changes in task and behavioral requirements or spatial context support the view that hippocampus is essential for learning new context-relevant information and for signaling changes in familiar contexts. The critical nature of this learned context information during navigation has been shown to include features of the external and internal environments (i.e. external sensory and intrinsic motivational information), and knowledge of the behavioral requirements of the task. This information is organized spatially and temporally, perhaps to facilitate efficient interactions with connected brain areas. Our results show that the economic condition or state likely comprises another type of information that contributes to the definition of recalled contextual memories that bias place field organization. Although the largest change in place field distributions occurred during the trials with the lowest probability of reward delivery, and these trials occurred at the end of recording sessions, it is not likely that unintentional electrode movement produced the observed effect. If that were the case, we should have observed significant place field reorganization for all trial types during the last block of recording. This was not the case: the pattern of place field re-distribution differed across win, lose and certain trial types, as we across free and forced choice condition during certain trials. Also, changing the economic conditions of the task did not alter the ability of place cells to exhibit clear and spatially-selective place fields. Therefore, it seems that altering economic conditions that biased choices resulted in dramatic redistribution of place fields relative to key task events (such as obtaining rewards) and the presentation of task-relevant information.

The strong impact of learned econometric features on hippocampal neural activity suggests that such information is an integral component of the memory that defines place field characteristics in familiar contexts. The economic state surrounding choice options may organize the matrix of sensory, motivational, and behavioral information that place fields are known to represent, and in

this way assist in the organization of adaptive place field sequences that may enable hippocampal-dependent memories (e.g., Lee and Wilson 2002; Foster and Wilson, 2006, Diba and Buzsaki, 2007; Johnson and Redish., 2007; Singer and Frank, 2007; Gupta et al., 2012). It is worth noting that responses of neurons of the midbrain-striatal-prefrontal decision making system are also modulated by the expected probability and magnitude of reward (Penner and Mizumori, 2012; Schultz et al., 1997; Lansink et al., 2009; Lisman and Redish, 2009; van der Meer and Redish, 2009; Schultz, 2007), but our data show that their modulation presents differently from hippocampal place fields or theta. **Figure 2.12** shows that unlike place cells and theta responses, reward prediction signals were generated by VTA neurons when rats performed the same task used to record our place fields. However, analogous to hippocampal place fields and theta, the VTA responses scaled to the expected probability of the reward. Further, similar to what has been shown in operant or Pavlovian paradigms (Schultz, 2007), the dopamine neurons in our study responded more strongly to reward predicting cues than to reward encounters per se (**Fig. 2.13**). Extrapolating from the prior dopamine studies, the pattern of dopamine cell firing that we observed illustrate that the task contingencies were well learned.

While our dopamine cell results are preliminary in that the data were collected from only two rats, our combined hippocampal and dopamine cell results is consistent with other evidence to support a view that the processing of economic information may functionally link hippocampal-based memory and decision systems of the brain. The dopaminergic processing of reward information that we observed with our maze-based task mirrors that observed in more traditional (non-hippocampal dependent) assessments of reward processing (Schultz, 2007). These dopamine reward signals are context-dependent in a manner similar to that observed for hippocampal place fields (e.g., Puryear et al., 2010). Further it is known that without VTA

function, hippocampal place fields are less stable (e.g., Martig et al., 2009). Analogous to the interpretation given for the enhanced dopamine response to rewards when they are the least expected (Fiorillo et al., 2003), one could interpret our observation of an overrepresentation of place fields at the goal during low probability win trials (**Fig. 2.7**) as signaling outcomes of greater surprise or salience. Such place field redistributions may have been signaled by dopamine responses to the reward predicting auditory cues. This place field salience signal may then update existing memories and attention mechanisms accordingly.

It is worth noting that the locus coeruleus also provides dopamine input to the hippocampus, and this input plays an important role in the hippocampal response to environmental novelty (Takeuchi et al., 2016). In the same study, optogenetic stimulation of the locus coeruleus enhanced memory in novel environments, and VTA inactivation did not attenuate the locus coeruleus stimulation-induced memory enhancement effect. Therefore, it was suggested that the locus coeruleus and VTA dopamine systems may make different functional contributions to hippocampal. Rather than coding environmental novelty, VTA dopamine may instead signal reward expectancy. This distinction is consistent with the findings of this study.

<u>Hippocampal theta dynamically reflects different types of econometric information during active navigation</u>

Theta power dynamically varied during task performance depending on the changing nature of salient economic information available to the rat (**Fig. 2.8**). These variations could not be accounted for by passage of time or sensory input since the changes in theta power did not correlate with specific behaviors, locations, reward or sensory input per se. This result illustrates that theta plays rather specific roles in information processing within hippocampus, perhaps to

coordinate neuronal responses according to the details of expected economic information. In this way, theta may effectively up- or down-regulate neural plasticity mechanisms as available information varies 'on-the-fly'. Indeed it is known that theta enhances synaptic plasticity mechanisms such as long term potentiation (Larson et al., 1986). Also, the proportion of place fields that showed phase precession increased as animals approached the location where they received information about the outcome of their choice. After receiving that information, the proportion of place fields that showed phase precession declined. Interestingly, this coincided with the time that theta power dramatically increased and place field reorganization around the goal location was observed. The increased theta around the goal location may reflect the occurrence of surprising, or low probability events that could trigger the necessary plasticity to update behavioral or neural states. In summary, the type of relationship between place fields and theta may vary according to the information that is currently available to, and being processed by, the animal.

The observed pattern of regulation of theta power by economic information is consistent with the hypothesis that in hippocampus, spatial context information is organized according to an understanding of choice options. Such an information framework could facilitate communication between other brain areas. For example, the reciprocally connected prefrontal cortex (Jay et al., 1989; Rajasethupathy et al., 2015) is known to be critical for adaptive response selection based on economic and predictive information (e.g., Pratt and Mizumori, 2001; Matsumoto et al., 2003; Baker and Ragozzino, 2014), and the prefrontal cortex and hippocampus show theta comodulation that coincides with decisions during spatial task performance (Jones and Wilson, 2005; Hyman et al., 2005). In the future, it will be of interest to determine if the economic regulation of hippocampal neural activity is abolished when the prefrontal cortex is taken off

line. Conversely, the predictive and behavioral planning functions of prefrontal codes may be disrupted if hippocampus is prevented from transmitting information about the surprising success of choices.

Another critical part of the decision neurocircuitry that may provide economic information to hippocampus is the midbrain dopamine system (Schultz, 2007). Manipulation of the dopaminergic system not only destabilizes place fields and spatial memory (Martig et al., 2009), but it also impairs hippocampal long term potentiation (Frey and Schroeder, 1990), and diminishes reward-place associations (McNamara, 2014). Thus the dopamine system may confer stability of reward processing, memory, and hippocampal physiology under stable context and task conditions. Indeed, reward related dopaminergic activity has been related to hippocampal memory processing (e.g., Wittman et al., 2005; McNamara et al., 2014). Dopamine activation may also induce neuroplasticity when needed, for example, when conditions change or are surprising. While we did not simultaneously record from the VTA and the hippocampus to evaluate their moment-to-moment correlation, our data (together with the existing literature) suggest that the dopamine system signals of upcoming rewards may have triggered the neuroplasticity needed for the place field reorganization observed after the cue was given. Consistent with this interpretation, dopamine responses to the reward predicting cue and place field reorganization toward the reward location scaled with the reward probability that was associated with a given trial. Also, dopamine cell response or place field reorganization was not observed when the rat did not receive reward predicting cues (i.e. on lose trials).

## Conclusion

The presents study reveals that the spatial organization of hippocampal place fields and theta power are strongly influenced by specific economic information that biases decisions and choices during goal-directed navigation. Moreover, dopamine cell responses that were recorded during the same task signaled upcoming rewards prior to the time when place field reorganization was observed. Thus, the dopamine system may contribute to the functional reorganization of place fields. In this way, behavioral economic information may exert control over hippocampal neural activity by providing a broad decision-based framework within which predicted spatial and temporal contextual information is interpreted and processed to form or update episodic memories.

# Chapter 3: A novel role for the periaqueductal gray in consummatory behavior

## Introduction

The periaqueductal gray (PAG) is a structure that plays an important role in endogenous analgesia, vocalizations, defensive behaviors, and autonomic regulation (Behbehani, 1995). Additionally, the PAG is connected to areas of the brain that are important for decision making (e.g. medial prefrontal cortex and amygdala, Beitz, 1982; Rizvi et al., 1991), reward processing and motivation (e.g. the ventral tegmental area, VTA, Omelchenko and Seasack, 2010), and basic homeostatic drives (e.g. the lateral hypothalamus and parabrachial nucleus, Behbehani et al., 1988; Krout et al., 1998). Because of these connections and the PAG's established role in a wide range of behaviors, we investigated whether the PAG is important in mediating appetitive processes.

When faced with competing behavioral options (i.e. to fight or to flee in the face of a threat, to forage or to hide when hungry in times of stress) animals must weigh the relative costs and benefits of each option. This essential process is mediated by a complex circuitry that engages multiple circuits of the brain. Based on its placement within decision-making, reinforcement learning, fear, and pain neurocircuitry, the PAG may serve to integrate threat and other noxious information with homeostatic and basic drives such as hunger in order to select the most appropriate behavior for the given situation. Indeed, it has been documented that expectancy of a food reward can activate endogenous opioid-mediated analgesia which is thought to be mediated by the PAG (Dum and Herz, 1984). It follows that reward expectation should elicit analgesia to allow an animal to ignore the pain and attend to a rewarding stimulus (Fields, 2007; Leknes and Tracey, 2008). Thus, the PAG may be recruited during appetitive processes.

The PAG sends excitatory and inhibitory projections to both GABAergic and dopaminergic VTA neurons (Omelchenko and Seasack, 2010) and provides considerable subcortical glutamatergic input to the VTA (Geisler et al., 2007; Sesack and Grace, 2010). The PAG is also known to directly mediate aspects of reward during drug reinforcement (Brandao, 1993) since mice will self-administer morphine into PAG (David and Cazala, 1994). In addition to being anatomically situated in reward circuitry, the PAG is also linked to the brain's core feeding circuit. However, stimulation of PAG-projecting hypothalamic AGRP neurons was not sufficient to induce feeding (Betley et al., 2013) and infusion of morphine into PAG inhibits lateral hypothalamus stimulation-induced feeding (Jenck et al., 1987). Thus, PAG activity may not directly induce feeding behavior, but instead may mediate whether approach behavior is appropriate given the current situation, such as when other behavioral drives like an exogenous threat or maternal drives are competing for an organisms' resources. Indeed, a previous study showed that the PAG was necessary for switching from maternal behavior to predatory insect hunting in the rat (Sukikara et al., 2006). Thus, the PAG should be investigated to assess if it is an important region for processing appetitive information that guides adaptive behaviors.

We hypothesized that the PAG could be involved in processing appetitive information. To investigate this, we temporarily inactivated the PAG using a GABA agonist while rats ran a maze-based foraging task for rewards. It was found that rats whose PAG was inactivated made significantly more errors, ate less of the reward, and showed decreased preference to first choose large rewards over small rewards. To directly address whether these observed effects were due to altered food consumption abilities, we conducted an additional PAG inactivation study during which we assessed rats' ability to consume food. We found that rats with an inactivated PAG showed significantly decreased food intake, even while hungry. We then directly measured

whether or not PAG neurons encode reward information by recording from PAG neurons in awake, behaving animals that also ran the foraging task. Indeed, we found strong, bidirectional, reward correlates in these neurons. These results, in conjunction with the anatomical evidence, suggest that the PAG may in fact play a role in reward related processing to regulate food intake.

**Methods**

*Subjects.* Twenty-three (9 to test PAG inactivation effects on maze performance; 7 to test PAG inactivation effects on food intake per se; 7 for PAG neural recordings;) male Long–Evans rats (340 – 460 g; Simonson Laboratories) were housed individually in Plexiglas cages. The rats were maintained on a 12 h light/dark cycle (lights on at 7:00 A.M.) and all behavioral experiments were performed during the light phase of the cycle. Each rat was allowed access to water ad libitum and food-deprived to 80-85% of its ad libitum feeding weight. Rats were handled and weighed daily. All animal care and use were conducted in accordance with University of Washington's Institutional Animal Care and Use Committee guidelines.

*Differential-reward, spatial memory task.* Detailed information of the apparatus and training procedures can be found in previous studies (Pratt and Mizumori, 2001; Puryear et al., 2010; Norton et al., 2011; Jo et al., 2013). Briefly, rats were familiarized with an elevated eight-arm maze (79 cm from the floor; **Fig. 3.1A**) and allowed to freely forage for sugar pellets (45mg sucrose tablets, TestDiet) scattered on black Plexiglas arms (58 x 5.5 cm each) that radiated from a circular central platform (19.5 cm in diameter) for 3 days. Each maze arm was hinged such that its proximal end to the central platform could be raised and lowered by remote control from an adjacent room. The maze was surrounded by black curtains on which hung several visual cues. Once the rat consistently moved about and consumed rewards on the maze, the training for a

spatial memory task started. While a rat was constrained to the central platform by lowering all maze arms (Fig. 1A), food cups located at the end of the maze arms were baited with either a large (4 pellets) or small (1 pellet) reward of 45mg sugar pellets on alternating arms (e.g., large rewards on even-numbered arms and small rewards on odd-numbered arms; counter-balanced across rats). Subsequent training trials consisted of a study and a test phase. During the study phase of each trial, four of the eight arms (two large reward and two small-reward arms) were randomly selected and presented individually. After presentation of the fourth arm, the test phase began upon making all maze arms accessible at once. The rat was required to collect the remaining rewards from the four arms not presented during the study phase. Revisits (i.e., when animals went at least halfway down a maze arm) to previously visited arms within a trial were defined as errors. When the animal returned to the central platform after visiting all eight arms, it was confined to the platform for an inter-trial interval (ITI) of 60 seconds. Meanwhile, all food cups were baited again and 10 trials, separated into two blocks of five trials, were given per day. The locations of differentially rewarded arms were held constant for each rat throughout training. After rats made an average of one or fewer errors per trial on a training day, rats underwent a surgical procedure for the implantation of recording electrodes or cannulae. Each rat was tested a total of four times: two saline sessions and two muscimol sessions.

For the neural recording study, the first block of 5 trials served as the baseline trials and was the same as described above. For the second block of 5 trials, one of three manipulations was administered: reward-switch, omission, or no manipulation. For the reward switch manipulation, the learned locations of the small and large rewards were switched. For the omission manipulation, two of the four arms visited during the study phase (one large and one small reward arm) had omitted rewards.

*Food intake tests*. For the first food intake test, rats were food restricted to 80-85% of their free-feeding weight and fasted for 24 hours prior to the food intake test. They had ad libitum access to water at all times. During the test, rats were randomly place in one of the four corners of the food intake arena (40 cm x 40 cm x 40 cm tall). In one of the corners was a plastic food cup filled with 1 gram regular rat chow pellets (Test diets filled cups that were affixed to the floor of the arena with Velcro.) Once rats were placed in the arena they had free access to the rat chow. After 10 minutes, the food cup was quickly replaced with a full one and weighed. This switching/weighing occurred again after 20, 30, 60, 90, and finally after 120 minutes. Rats were continuously monitored and tracked using ANY-maze software and a camera mounted above the arena. After two hours had expired, the rat was removed from the arena and returned to its home cage. Any food particles left in the arena were collected and weighed to reflect accurate food consumed amount. The arena and food cup was cleaned with Virkon (1%) solution between rats.

The second food intake test quantified the latency to consume a palatable reward. As with the first food intake test, rats were maintained at 80-85% free feeding weight and fasted 24 hours prior to the sucrose test. Rats were tested in the same arena as the first food intake test. In the center of the arena a food cup was affixed to the arena floor with Velcro. Rats were placed in the arena and habituated for 2 minutes prior to the beginning of the first trial. To begin, four 45mg sugar pellets were placed in the food cup. The latency to consume all four sugar pellets was recorded. Once the rat consumed all pellets, the ITI of 60 seconds began. Rats were given 120 seconds to consume the four pellets before they were taken away and the next ITI began. Fresh pellets were given at the beginning of each trial even if the rat failed to consume them in the previous trial. A session consisted of 20 trials.

*Electrode, cannula, and surgical procedures*. Recording tetrodes were constructed from 20 µm lacquer-coated tungsten wires (California Fine Wire) and mounted on an array of three or four independently adjustable in-house made microdrives (2 or 3 tetrodes per microdrive). Tetrode tips were gold-plated to reduce impedance to 0.1– 0.4 MΩ (tested at 1 kHz). The guide cannulae (Plastics One, Roanoke, VA) were composed of 26 gauge stainless steel tubes cut to custom length (see below) whereas the injection cannulae were 33 gauge, cut to extend 1.0mm below the guide cannulae. Each rat was placed in an induction chamber and deeply anesthetized under isoflurane (4% mix with oxygen at a flow rate of 1 L/min). Under deep anesthesia, the animal was placed in a stereotaxic instrument (David Kopf Instruments) and anesthesia was maintained throughout surgery by isoflurane (1-2.5%) delivered via a nosecone. The skull was exposed and adjusted to place bregma and lambda on the same horizontal plane. After small burr holes were drilled, two 25 gauge cannulae or the microdrive were bilaterally implanted into the PAG (6.0 mm posterior, 0.5 mm lateral, and 6.0 mm ventral to bregma). The cannulae bilaterally targeted the PAG while the microdrive array was unilaterally implanted. Cannulae and microdrive arrays were secured in place with anchoring screws and dental cement. A 33 gauge dummy cannula was inserted into each guide to prevent clogging. Rats were allowed to recover for 7 days, during which they were weighed and handled daily.

*Intracranial microinjection*. Muscimol (a $GABA_A$ agonist; 1 µg/µl dissolved in 0.9% saline) was used to temporarily inactivate the PAG. Microinjection procedures were performed as previously described (Jo et al., 2013). Briefly, a 33 gauge injection cannula extending 1 mm below the tip of the guide cannula was connected to a 10 µl syringe (Hamilton) via polyethylene tubing (PE 20). Prior to tests days for the maze and food intake tests, each rat received an injection of 0.9% saline to habituate them to the injection protocol. On test days, 0.1-0.3 µl/side of either muscimol

or saline was injected bilaterally at a rate of 12 µl/h using an infusion pump (KD Scientific). The injection cannulae were left in place for an additional 1 min to allow diffusion of the drugs from the injection tip. Rats were then returned to their home cages, and were closely observed for 10-20 min before they were placed on the maze or in the food intake test box. Drug was injected into the PAG before the second block of five trials on the maze. Saline or muscimol was injected in random orders across rats.

*Single-unit recording and postsurgical procedures.* After a week of recovery, rats were returned to a food-restricted diet and spontaneous neural activity in the PAG was monitored as follows: the electronic interface board (Neuralynx) of the microdrives was connected to preamplifiers, and the outputs were transferred to a Cheetah data acquisition system (Neuralynx). Signals were filtered between 0.6 and 6 kHz, and digitized at 16 kHz. Neuronal spikes were recorded for 2 ms after the voltage deflection exceeded a predetermined threshold at 500 –7000X amplification. If no units were encountered, tetrodes were lowered in 40 µm increments to target new units. A video camera mounted on the ceiling tracked infrared LED signals attached to the preamplifier and subsequent position data were relayed to the acquisition system. Once clearly isolated and stable units were found, recording on the maze began. Experimental sessions continued until tetrodes passed through the PAG based on the distance traveled from the brain surface.

*Histology.* After the completion of all recording sessions, cannula positions and tetrode locations were verified. Rats were deeply anesthetized under 4% isoflurane. For neural recordings, the final position of each tetrode was marked by passing a 15A current through a subset of the tetrode tips for 15 s. Then, the animals were given an overdose of sodium pentobarbital and transcardially perfused with 0.9% saline and a 10% formaldehyde solution. Brains were stored in a 10% formalin–30% sucrose solution at 4°C for 72 h. The brains were frozen, and then cut in

coronal sections (45µm) on a freezing sliding microtome. The sections were then mounted on gelatin-coated slides, stained with cresyl violet, and examined under light microscopy. Only cells verified to be recorded in PAG were included in the data analysis and animals with both cannulae in the PAG were included in the behavioral analysis in the inactivation studies.

*Data analysis*. PAG single units were isolated using an Offline Sorter (Plexon). Various waveform features, such as the relative peak, valley, width, and principle component, were compared across multiple units simultaneously recorded from the four wires of a tetrode. Only units showing good recording stability across blocks were included. Further analysis of the sorted units was performed with custom Matlab software (Mathworks). To examine the reward-related responses of PAG neurons, peri-event time histograms (PETHs) were constructed at 4.0 s around the time of all reward acquisition-triggered events. A bin size of 50 ms was used for all PETHs. Reward-related PAG cells were identified based on phasic responses to reward acquisition (Martig and Mizumori, 2011; Jo et al., 2013). A PAG neuron was considered reward responsive if it had significantly elevated or decreased firing during the 500 ms after obtaining a large reward as measured by a Wilcoxon signed rank test comparing the firing during this period to a non-rewarded period on the maze. Based on these results, neurons were grouped into reward-inhibited, reward-excited, or not reward modulated. Subsequently, the firing rate patterns of those same cells were assessed relative to encounters with small rewards to determine whether there were differences in firing depending on reward magnitudes.

*Statistical analysis*. Statistical analyses were performed using SPSS 19.0, Graphpad Prism 6.0, or custom Matlab software (Mathworks). Drug effects on behavioral performance on the radial maze were analyzed with dependent t tests (a rat's drug performance was compared to saline performance, normalized to baseline performance) for pellet consumption and errors. Preference

for large reward was assessed with a multifactorial ANOVA (repeated measures for choice and drug group as another within subjects factor) followed by Bonferroni's post hoc pairwise comparisons. Drug effects on the food intake tests were analyzed with dependent t tests (rats' food intake, movement variables, or latency to consume sucrose was compared for saline and muscimol). Cumulative food consumed was assessed with repeated measures ANOVA.

Neural responses across blocks (for reward switch conditions) or reward encounter type (large, small or no reward) were analyzed with a two way repeated-measures ANOVA, followed by Bonferroni's post hoc pairwise comparisons. Two way ANOVA (block as within subjects' factor, manipulation type as between subjects' factor) was used to assess neural responses for the reward switch conditions. These tests were performed separately for each reward response group, i.e. excited, inhibited, or not modulated by reward. Pearson's correlation tests were used to assess relationships between firing rate and velocity. Two-tailed p values of $\leq 0.05$ were considered statistically significant, unless otherwise noted. All data are expressed as mean ±SEM, unless otherwise noted.

**Results**

*PAG inactivation effects on radial maze performance*

Our initial investigation into whether or not PAG plays a role in reward processing was assessed by temporarily inactivating the PAG with muscimol, a $GABA_A$ receptor agonist, while rats ran on a radial eight arm maze to collect rewards (sugar pellets) (**Fig. 3.1A**). $GABA_A$ receptors are present throughout the entire periaqueductal gray matter of the rat (Griffiths and Lovick, 2005). Using the same task, previous research has shown that inactivating the VTA resulted in compromised working memory performance (Martig et al., 2009; Martig and Mizumori, 2011).

Because of its anatomical connection with VTA, we hypothesized we would also see compromised working memory performance when inactivating PAG because the appropriate computations necessary for optimal performance in regards to working memory and incentive salience (Berridge and Robinson, 1998) in areas such as VTA would be compromised. We also hypothesized that if PAG is necessary for processing information about the presence of rewarding stimuli, the consumption of rewards would diminish when normal PAG function was temporarily halted. We measured the influence on behavior by comparing the number of errors the rats made, the number of pellets they ate, and their preference for a large or small reward in baseline, vehicle, and inactivation conditions. It was found that when compared to vehicle injection session, PAG inactivated rats made significantly more errors (dependent t test, saline vs muscimol normalized to baseline errors, $t$ (1, 17) = 5.46, $p < 0.001$) (**Fig. 3.1D**) and they ate less of the reward (dependent t test, saline vs muscimol normalized to baseline pellets; $t$ (1, 17) = 5.59, $p < 0.001$) (**Fig. 3.1C**). When comparing the preference to retrieve large rewards during the test phase of each trial, it was found that muscimol-treated rats showed a significantly decreased preference to choose large rewards first (two way repeated measures ANOVA, main effect of treatment: $F$ (1, 7) = 23.19, $p < 0.001$; main effect of choice order: $F$ (3, 21) = 61.47, $p < 0.001$; significant interaction between treatment and choice order: $F$ (3, 21) = 23.44, $p < 0.001$) (**Fig. 3.1E**). Further it was found that when comparing the first four choices during the test phase individually, muscimol treated rats showed a consistently lower overall preference for large rewards except for on the fourth choice (Bonferroni post-hoc comparisons, all $p < 0.05$ except fourth choice) (**Fig. 3.1E**). These results, taken together with the anatomical evidence, suggest that the PAG may in fact play a role in reward related processing.

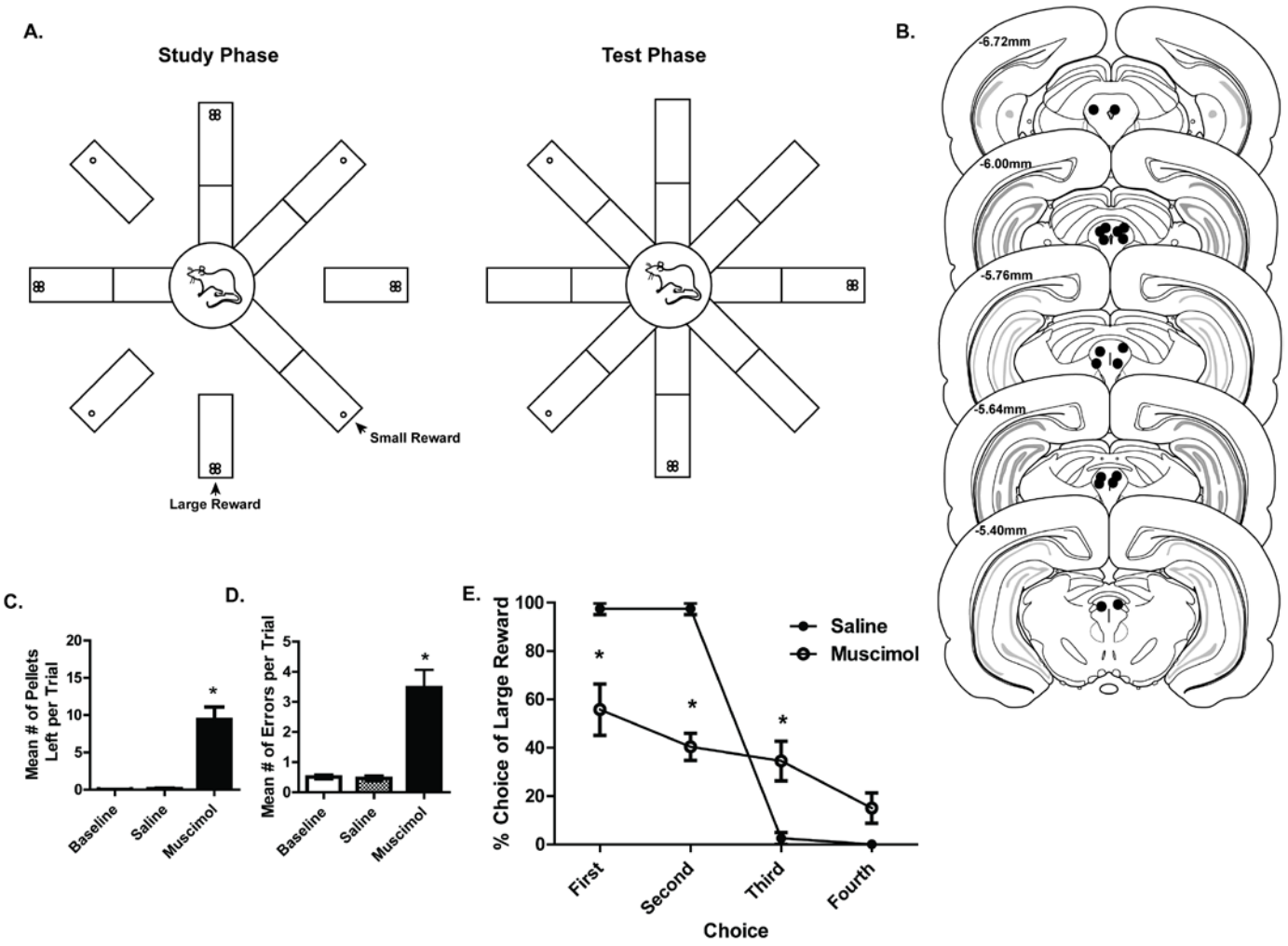



**Figure 3.1. A.** Schematic of the radial arm maze task. During the study phase, 1 arm of the maze was raised at a time until four arms were visited by the rat (2 large reward arms and 2 small reward arms). Large rewards (4 sugar pellets) were on every other arm of the maze. Each rat was tested a total of four times: two saline sessions and two muscimol sessions. During the test phase, all 8 arms of the radial maze were raised and rats were free to visit any arm of their choice to collect the rewards from the remaining 4 arms. **B.** Reconstruction of bilateral cannulae tip placement relative to Bregma (N = 9). **C.** Mean number of pellets left per trial out of the available 20 pellets during baseline, saline, and muscimol conditions. There were significantly more pellets left on muscimol trials compared to baseline and saline (dependent t test, saline vs muscimol normalized to baseline pellets; $t$ (1, 17) = 5.59, $p < 0.001$). **D.** Mean number of errors per trial during baseline, saline, and muscimol conditions. There were significantly more errors on muscimol trials compared to baseline and saline (dependent t test, saline vs muscimol normalized to baseline errors, $t$ (1, 17) = 5.46, $p < 0.001$). **E.** Preference to visit the large reward arms first during the test phase of a trial for saline- and muscimol-treated rats. Muscimol-treated rats had significantly reduced preference to collect large rewards first compared to saline-treated rats (two way repeated measures ANOVA, main effect of treatment: $F$ (1, 7) = 23.19, $p < 0.001$; main effect of choice order: $F$ (3, 21) = 61.47, $p < 0.001$; significant interaction between treatment and choice order: $F$ (3, 21) = 23.44, $p < 0.001$). All error bars represent ±SEM.

*PAG inactivation effects on food intake*

While intriguing, the radial maze inactivation study data does not directly support the conclusion that PAG is necessary for reward consumption per se as the requirement of animals to complete a complex spatial working memory task introduces multiple alternative explanations for the results observed. To test some of these alternative explanations, an additional inactivation study was performed with a separate group of rats. The purpose of this study was to more directly assess if PAG is necessary for food consumption. Histological reconstructions show that cannula tips were not in exactly the same location of the PAG for each rat (**Fig. 3.2A**). Previous research has shown functional differences for each column of the PAG. Thus, rats were grouped by column of the PAG that was targeted. Since no significant behavioral differences were observed (data not shown), animals' data were grouped together for subsequent analyses.

PAG inactivation significantly reduced the total amount of food consumed over the two hour food intake test compared to vehicle treated rats ($t$ (6) = 2.95, $p$ = 0.01) (**Fig. 3.2C**). Another way to look at these data was to investigate differences in cumulative food intake over time. Not surprisingly, there was a main effect of time (two way repeated measures ANOVA, $F$ (5, 30) = 21.23, $p < 0.001$), no main effect of treatment group ($F$ (1, 6) = 4.37, $p$ = 0.08), but a significant interaction between time and treatment group ($F$ (5, 30) = 22.24, $p < 0.001$) (**Fig. 3.2D**). Examining each time point individually reveals that the two treatment groups were only significantly different ($p < 0.05$) towards the end of the testing period, i.e. at the 90 minute and 120 minute time points. Thus while the PAG inactivated animals were still able to consume food, the total amount of food consumed was significantly diminished compared to vehicle treatment

especially at the end of the test period. It is possible that rats ingested less food because of a general motor impairment. To address this, movement data collected during the two hour food intake test were analyzed to see if muscimol treated rats had significant impairments in general locomotor behavior. When assessing various measures of locomotion, it was found that this was not the case. In fact, muscimol treated rats showed increased locomotion relative to saline on measures in terms of total time mobile ($t$ (6) = 2.71, $p$ = 0.04), and while not significant, also showed a slight increase in mean speed and total distance traveled (**Fig. 3.2E-F**).

The latency to consume sucrose test revealed a similar impairment in food consumption for PAG inactivated rats (**Fig. 3.2G**). The average time it took a muscimol treated rat to consume four 45mg sugar pellets was significantly increased relative to vehicle treated trials ($t$ (6)= 3.37, $p$ = 0.01) (**Fig. 3.2H**). The results provide evidence that PAG is needed for normal consummatory behaviors for both regular food and palatable rewards.

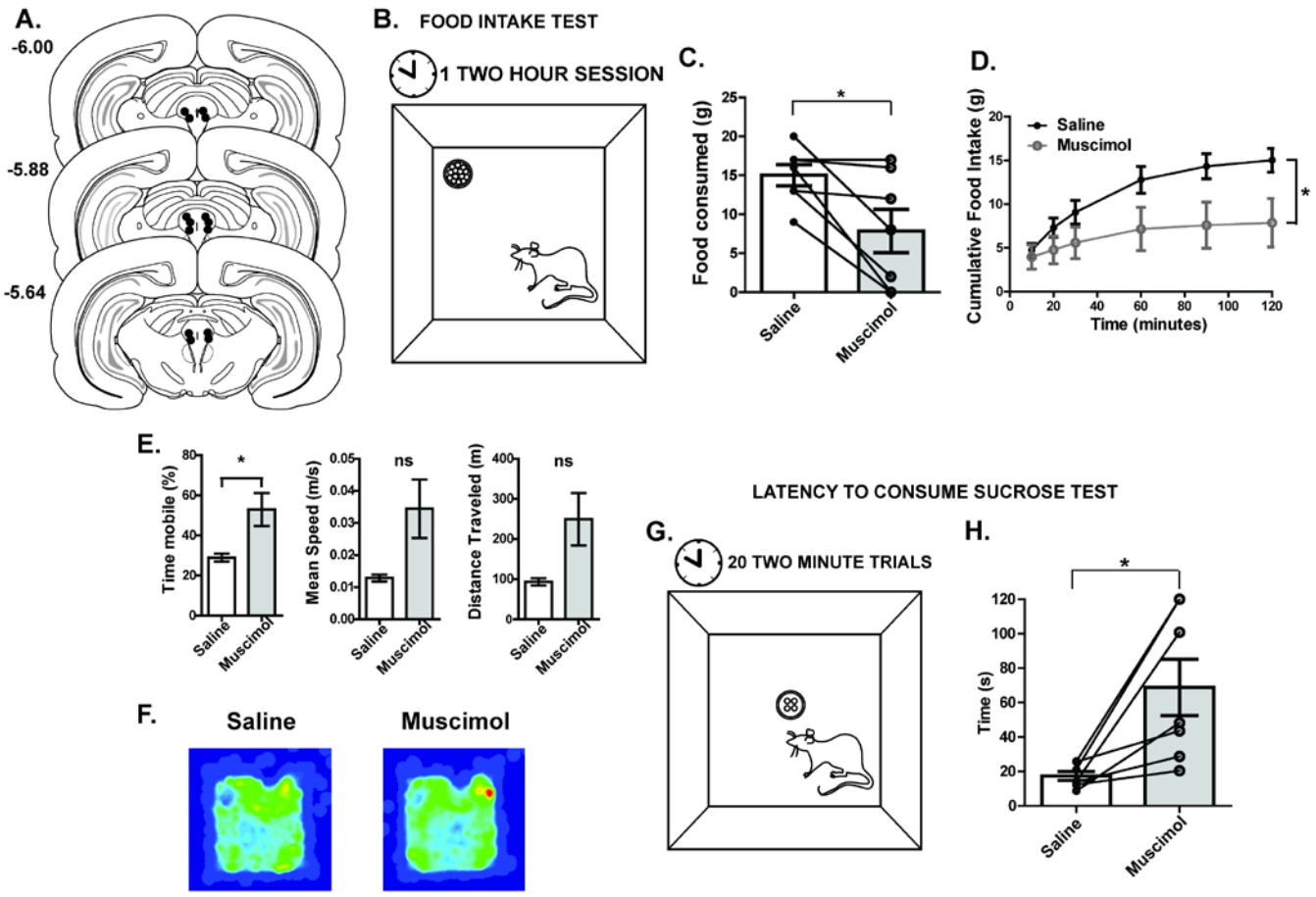



**Figure 3.2. A.** Reconstruction of bilateral cannulae tip placement relative to Bregma (N = 7) targeting the PAG. **B.** Food intake test: rats were fasted for 24 hours prior to the test. During the 2 hour test, rats were placed in the arena (40 x 40 x 40cm). A plastic food cup was filled with 1 gram regular rat chow pellets. Food was weighed at 20, 30, 60, 90, and 120 minutes. Rats were tracked using ANY-maze software. Any food particles left in the arena were collected, weighed, and subtracted from total food consumed. **C.** Food intake test results: Following temporary inactivation of the PAG, the total amount of food consumed over two hours was significantly decreased compared to vehicle treated rats ($t$ (6) = 2.95, $p$ = 0.01). **D.** PAG inactivation produced changes in cumulative food intake over time. There was a main effect of time (two way repeated measures ANOVA, $F$ (5, 30) = 21.23, $p < 0.01$), no main effect of treatment group ($F$ (1, 6) = 4.37, $p$ = 0.08), but a significant interaction between time and treatment group ($F$ (5, 30) = 22.24, $p < 0.01$). The two treatment groups were significantly different ($p < 0.05$) towards the end of the testing period, at the 90 and 120 minute time points. **E.** Movement data collected during the two hour food intake test revealed that reduced consumption was not due to less activity. Muscimol treated rats showed increased locomotion relative to saline on measures in terms of total time mobile ($t$ (6) = 2.71, $p$ = 0.04), and while not significant, also showed an increase in mean speed and total distance traveled. **F.** Average occupancy data over the two hour food intake test for each group of rats. The total time in various arena locations were similar between saline- and

muscimol-treated rats. **G.** Latency to consume sucrose test: four 45mg sugar pellets were placed in the food cup. The latency to consume all four sugar pellets was recorded. Once the rat consumed all pellets, the ITI of 60 seconds began. Rats were given 120 seconds to consume the four pellets before they were taken away and the ITI began. A session consisted of 20 trials. **H.** Latency to consume sucrose after PAG inactivation: Individual points represent rats' mean latency to consume sucrose pellets over 20 trials. The time it took muscimol treated rats to consume four 45mg sugar pellets was significantly increased relative to vehicle treated trials ($t(6)= 3.37$, $p = 0.01$). All error bars represent ±SEM.

*PAG neural activity during maze performance*

A total of 237 individual PAG neurons were recorded from 7 rats as they ran on a spatial working memory maze task. Of particular interest was whether or not these neurons would show distinct neural correlates with reward encounters. Thus, neurons were first sorted into one of three groups based on their phasic responses to reward encounters: reward excited (n = 93), reward inhibited (n = 58), or not significantly reward modulated (n = 86) (**Fig. 3.3D-F**) (see methods). Then, whether or not reward magnitude had any effect on these phasic reward-induced responses was assessed by comparing each neuron's firing rate during the time just before (two 250ms epochs, -500ms to -250ms; -250ms to 0ms) and after (two 250ms epochs, 0ms to 250ms; 250ms to 500ms) reward encounters for large rewards, small rewards, and no rewards (errors). This analysis allowed us to assess if time (centered on reward encounters) had a significant effect on the firing rate of these neurons as well. For the reward excited neurons, there was a significant main effect of reward type (Two way repeated measures ANOVA, $F$ (2,180) = 21.23, $p < 0.001$) time ($F$ (3,270) = 5.23, $p = 0.002$), and a significant interaction between reward and time ($F$ (6,540) = 11.17, $p < 0.001$) (**Fig. 3.3G-H**). Post-hoc pairwise comparisons (comparing all combinations of pairs, i.e. large reward with small, small with errors, and large with errors) with a Bonferroni adjustment for multiple comparisons for the significant main effect of reward show that there were significant differences comparing all levels of reward type ($p < 0.001$ for all comparisons). The firing rate in response to large rewards was greater than that of small rewards and no rewards, and small rewards elicited a higher firing rate than no reward. Post-hoc pairwise comparisons for the significant main effect of time (comparing all combinations of pairs, i.e. the first epoch with the second, the first epoch with the third, and so on) revealed that only the first time epoch (-500ms to -250ms) was significantly different from the second (-250ms to 0ms; $p =$

0.01) and third epoch (0ms to 250ms; $p = 0.01$) but not compared to the fourth epoch (250ms to 500ms, $p = 0.26$). In other words, during the time directly preceding and directly after the reward encounter, reward excited neurons showed significantly increased firing rate compared to at least 250ms preceding a reward encounter. The fact that these peri-reward time periods were not significantly different from the fourth time epoch (250ms to 500ms) suggests that excitatory response was sustained. It is also important to note that the excitation began directly preceding reward encounters, i.e. the second epoch (-250ms to 0ms), likely reflecting anticipation or sensory information indicating reward was imminent. This analysis shows that for neurons classified as reward excited, reward magnitude and time from reward had a significant influence on PAG neurons' firing rate, supporting the hypothesis that these factors are also encoded by neurons that were already classified as having significantly elevated rates in response to reward encounters.

The same analysis was repeated for the reward inhibited neurons, which revealed a significant main effect of reward magnitude (Two way repeated measures ANOVA, $F$ (2,108) = 17.47, $p <$ 0.001) time ($F$ (3,162) = 15.11, $p < 0.001$), and a significant interaction between reward and time ($F$ (6,324) = 6.82, $p < 0.001$) (**Fig. 3.3I-J**). Post-hoc pairwise comparisons with a Bonferroni adjustment for multiple comparisons for the significant main effect of reward showed that firing rates in response to large and small rewards were not significantly different ( $p = 0.06$). However, neurons' responses to errors were significantly reduced, as the magnitude of inhibition was not as great, compared to the inhibition observed for neural responses to both large reward encounters ($p < 0.001$) and small reward encounters ($p < 0.001$). Post-hoc pairwise comparisons for the significant main effect of time revealed that all pairwise comparisons for the different time points were significantly different ($p < 0.05$) except for when comparing the third time

epoch (0ms to 250ms) to the last time epoch (250ms to 500ms; $p = 0.67$). In other words, the firing rate of reward inhibited neurons was significantly reduced during reward encounters compared to pre-reward encounters, and the degree of firing rate inhibition was maintained for at least 500ms post-reward encounter. Interestingly, because the first (-500ms to -250ms) and second time epoch (-250ms to 0ms) were also significantly different from each other, in that the firing rate was already reduced in the second time epoch compared to the first, this shows that inhibition of these neurons begins just prior to actual reward encounters. This could reflect anticipation or expectation, which is addressed below. This analysis shows that for reward inhibited neurons, time from reward encounter and presence of reward (rather than reward magnitude) is also encoded in these neurons' firing rates.

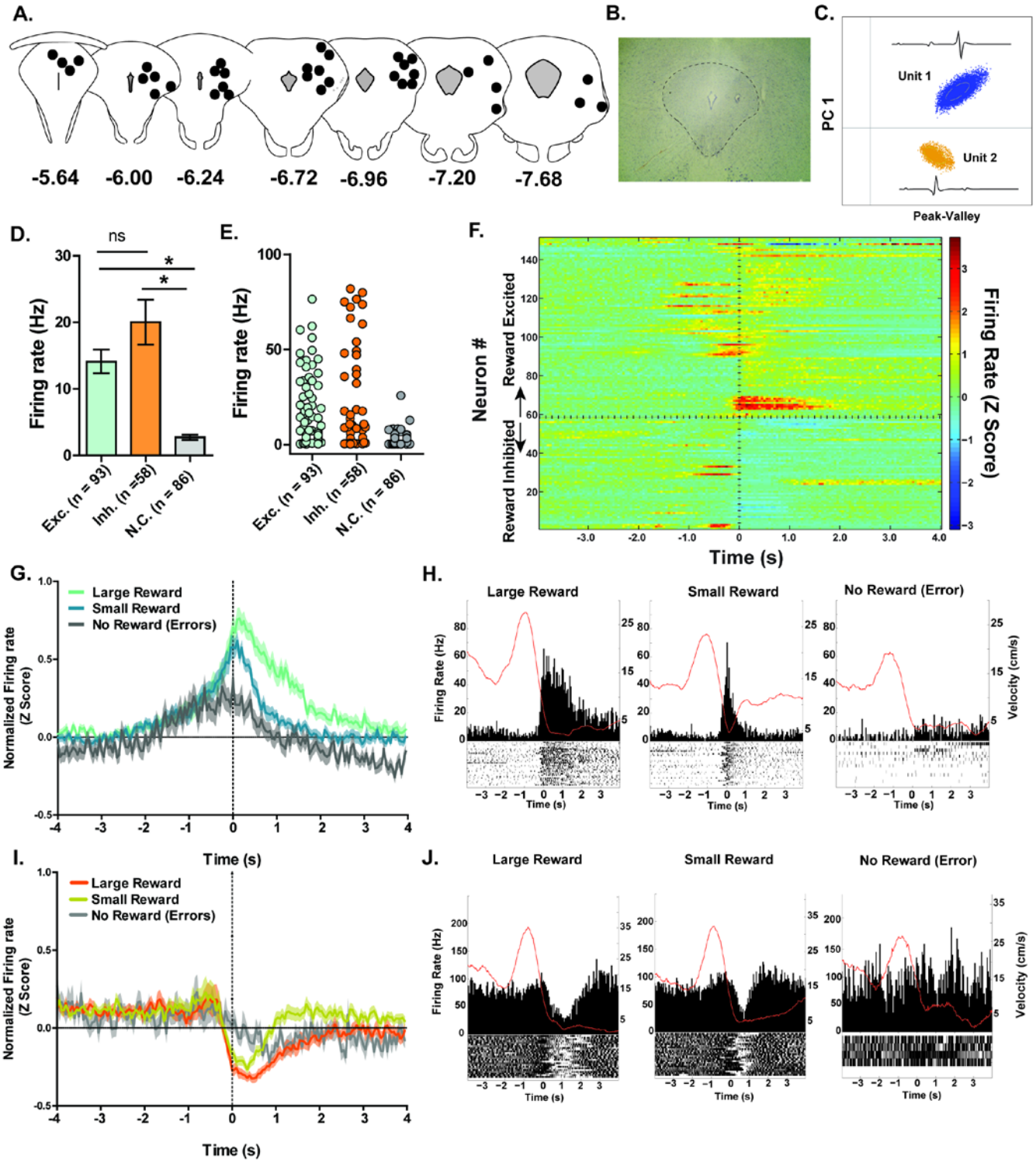

A.
-5.64
-6.00
-6.24
-6.72
-6.96
-7.20
-7.68
B.
C.
Unit 1
Unit 2
PC 1
Peak-Valley
D.
ns
*
*
Firing rate (Hz)
Exc. (n = 93)
Inh. (n =58)
N.C. (n = 86)
E.
Firing rate (Hz)
Exc. (n = 93)
Inh. (n =58)
N.C. (n = 86)
F.
Neuron #
Reward Excited
Reward Inhibited
Firing Rate (Z Score)
Time (s)
G.
Large Reward
Small Reward
No Reward (Errors)
Normalized Firing rate (Z Score)
H.
Large Reward
Small Reward
No Reward (Error)
Firing Rate (Hz)
Velocity (cm/s)
Time (s)
I.
Time (s)
Large Reward
Small Reward
No Reward (Errors)
Normalized Firing rate (Z Score)
Time (s)
J.
Large Reward
Small Reward
No Reward (Error)
Firing Rate (Hz)
Velocity (cm/s)
Time (s)

**Figure 3.3 A.** Reconstruction of terminal tetrode locations relative to Bregma (mm). Black dots represent the location of the electrolytic lesion that marks the tetrode location. **B.** Example electrolytic lesion in the PAG. **C.** Illustration of signals from two simultaneously recorded PAG neurons**. D.** A total of 237 individual neurons from 7 rats were recorded from the PAG during spatial working memory task performance (see **Fig. 1A**). Individual neurons were first sorted into one of three groups based on their phasic responses to reward encounters: reward excited, reward inhibited, or not significantly reward modulated (Wilcoxon signed rank test). Reward modulated neurons had significantly higher firing rates than non-reward modulated cells (one way ANOVA, $F$ (2, 24) = 21.08, $p < 0.0001$; Bonferroni's post-hoc comparisons to compare each pair, * indicates $p < 0.05$). Exh. = Reward Excited, Inh. = Reward Inhibited, N.C. = not correlated to reward. Error bars represent ±SEM. **E.** Each dot represents the mean firing rate of an individual neuron from each group. **F.** Heatmap of normalized firing rates of all reward responsive neurons. Time 0 indicates onset of large reward encounters. Dotted line separates the reward excited and reward inhibited groups. As observed, some reward responsive neurons showed changes in firing patterns before reward onset began while others increased or decreased firing after reward encounters. **G.** Normalized firing rates of reward excited neurons in response to large, small, and no rewards. Time 0 indicates onset of reward encounters for large and small rewards or expected reward location for error trials. The solid line indicates mean firing rate within 50ms time bins; the shaded region around each line represents ±SEM. The magnitude of reward responses scaled with reward magnitude (see Results). **H.** Peri-event time histograms (PETHs) of a reward excited neurons' responses to large, small, and no rewards. Left Y axis represent the mean firing rate (Hz) over all events; right Y axis is mean velocity (cm/s), represented by the red line. Time 0 indicates onset of reward encounters for large and small rewards or expected reward location for error trials. **I.** Normalized firing rates of reward inhibited neurons in response to large, small, and no rewards. Time 0 indicates onset of reward encounters for large and small rewards or expected reward location for error trials. The solid line indicates mean firing rate within 50ms time bins; the shaded region around each line represents ±SEM. **J.** PETHs of a reward-inhibited neurons' responses to large, small, and no rewards. Left Y axis represent the mean firing rate (Hz) over all events; right Y axis is mean velocity (cm/s), represented by the red line. Time 0 indicates onset of reward encounters for large and small rewards or expected reward location for error trials.

It is important to note that while PAG neurons could be functionally subdivided into three separate groups based on their time-locked responses to rewarding stimuli, within each subgroup there was a marked heterogeneity in the types of responses observed. For example, within the reward excited neuron group, some neurons showed a sustained phasic excitation to rewards that was initiated before reward encounters, whereas other neurons only showed reward-correlated excitation once the reward location was reached. It could be that the former set of neurons responded to sensory cues that indicated that a sugar encounter is imminent, such as olfactory cues. Interestingly, while there was a lot of variance in the firing rate across individual neurons, the mean rate of the neurons that did not respond to reward encounters was significantly lower than that of the reward correlated neurons (one way ANOVA, $F$ (2,235) = 21.08, $p < 0.001$) while the firing rates of excited and inhibited neurons were not significantly different from each other (**Fig. 3.3D-E**). It could be that the different subgroups of reward-responsive neurons are comprised of distinct neuronal population (e.g. interneurons vs projection neurons), but future work is needed to delineate whether or not this is the case.

Because PAG neurons send projections to regions of the brain that show reward prediction errors, such as ventral tegmental area dopamine neurons, we were interested to investigate whether or not expectation of rewards, and more specifically, violation of those expectations, would be reflected in PAG neural responses. To examine this, we investigated data taken from sessions during which rats underwent the reward switch manipulation for the second half of the session, i.e. no manipulation of reward location during block 1 followed by switching the placement of large and small rewards during block 2. During these sessions, the locations of large and small rewards were switched for the second half of trials. Because rats were very familiar with the placement of these rewards (as evidenced by their preference to visit large

rewards first), it follows that they have a reward size expectation when making particular choices and that smaller-than or larger-than-expected rewards would produce a reward prediction error signal. Indeed, previous research has shown that VTA neurons have significantly increased firing to larger than expected rewards when using this same task design (Puryear et al., 2010; Jo et al., 2013). Thus, to test if violation of expectation of reward size was reflected in PAG neurons reward responses, a two way ANOVA for manipulation type (none or reward switch) and block (1 and 2) were performed separately for small and large rewards on the normalized firing rates during the first 500ms after reward encounters (**Fig. 3.4**). For reward excited neurons, the responses to large reward were not significantly affected by order (aka block; $F$ (1, 93) = 0.16, $p$ = 0.69) or manipulation ($F$ (1, 93) = 0.94, $p$ = 0.34) (**Fig. 3.4A,C**). The results were similar for responses to small rewards, as block ($F$ (1,100) = 0.32, $p$ = 0.89) and manipulation ($F$ (1,100) = 0.37, $p$ = 0.54) did not significantly affect normalized firing rate (**Fig. 3.4B,D**). For reward inhibited neurons, similar results were observed for both large reward (no significant effects of block ($F$ (1, 57) = 0.11, $p$ = 0.74) or manipulation ($F$ (1, 57) = 1.38, $p$ = 0.25)) (**Fig. 3.4E,G**) and small rewards (block: ($F$ (1, 49) = 0.01, $p$ = 0.91), manipulation: ($F$ (1, 49) = 0.00, $p$ = 0.99)) (**Fig. 3.4F,H**). These data support the conclusion that PAG neurons' response to reward encounters does not reflect expectation, but instead the magnitude of response (whether inhibition or excitation) is governed by the magnitude of reward presented to the animal at that time.

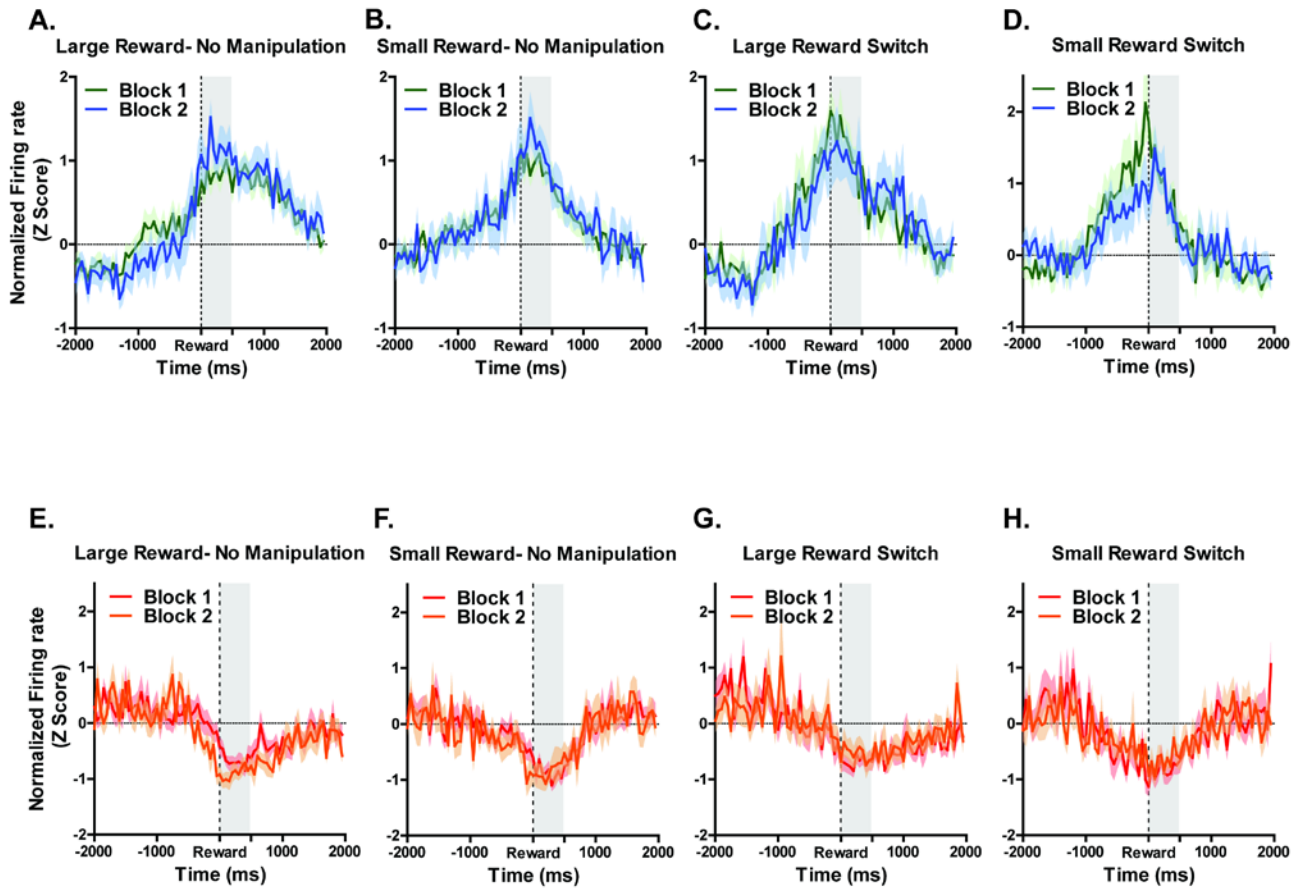



**Figure 3.4.** For all figures, the Y axis represents the normalized firing rate during a 4 second window centered on reward encounters. X axis represents time. Block 1 is the first 5 trials during which sizes of rewards were always located as expected. During Block 2, rewards could remain in their expected location (no manipulation) or could be switch (reward switch condition). For "large reward switch", during Block 2, rats received a large reward when they were expecting a small reward. For "small reward switch", during Block 2, rats received a small reward when they were expecting a small reward. A two way ANOVA for manipulation type (none or reward switch) and block (1 and 2) were performed separately for small and large rewards on the normalized firing rates during the first 500ms after reward encounters (shaded region). **A.** Normalized responses of reward excited neurons to large rewards when there was no manipulation in Block 2. **B.** Normalized responses of reward excited neurons to small rewards when there was no manipulation in Block 2. **C.** Normalized responses of reward excited neurons to large rewards when reward locations were switched in Block 2. **D.** Normalized responses of reward excited neurons to small rewards when reward locations were switched in Block 2. **E.** Normalized responses of reward inhibited neurons to large rewards when there was no manipulation in Block 2. **F.** Normalized responses of reward inhibited neurons to small rewards when there was no manipulation in Block 2. **G.** Normalized responses of reward inhibited neurons to large rewards when reward locations were switched in Block 2. **H.** Normalized responses of reward inhibited neurons to small rewards when reward locations were switched in Block 2. Block order and manipulation did not significantly affect firing rates during reward

encounters for small or large rewards for either reward excited or reward inhibited neurons (all *p's* > 0.05; see Results).

It was found that the firing rates of 38% (n = 91) of recorded neurons were significantly correlated with the rats' velocity of movement across the maze ($p \leq 0.05$) (**Fig. 3.5A**). The majority of velocity correlated neurons were negatively correlated (negatively correlated: 67%, n = 61, mean $R = 0.78 \pm 0.2$; positively correlated: 33%, n = 30, mean $R = 0.77 \pm 0.02$) (**Fig. 3.5A**). Of those significantly correlated to velocity, 62 (69%) were also significantly correlated with reward. Dual encoding of both movement and reward has been previously found in other midbrain regions such as the VTA (Puryear et al., 2010) and LDTg (Redila et al., 2015). To assess velocity's contribution to changes in firing rate, we fit a linear equation of each neuron with velocity as the predictor during a window where the rats were not getting rewarded. Then, we calculated the difference between the expected firing rate and the observed firing rate (residual) during the period where the rat was getting rewarded (**Fig. 3.5C**). Residuals close to 0 indicate firing rate does not deviate from velocity-predicted values. Interestingly, some velocity correlated neurons did not have residuals close to 0, suggesting that in these neurons there is conjunctive encoding of both movement and reward information. We also separated correlated neurons by rat and found that all rats had more reward correlated than velocity correlated neurons, although the exact proportion did vary from rat to rat (**Fig. 3.5D**).

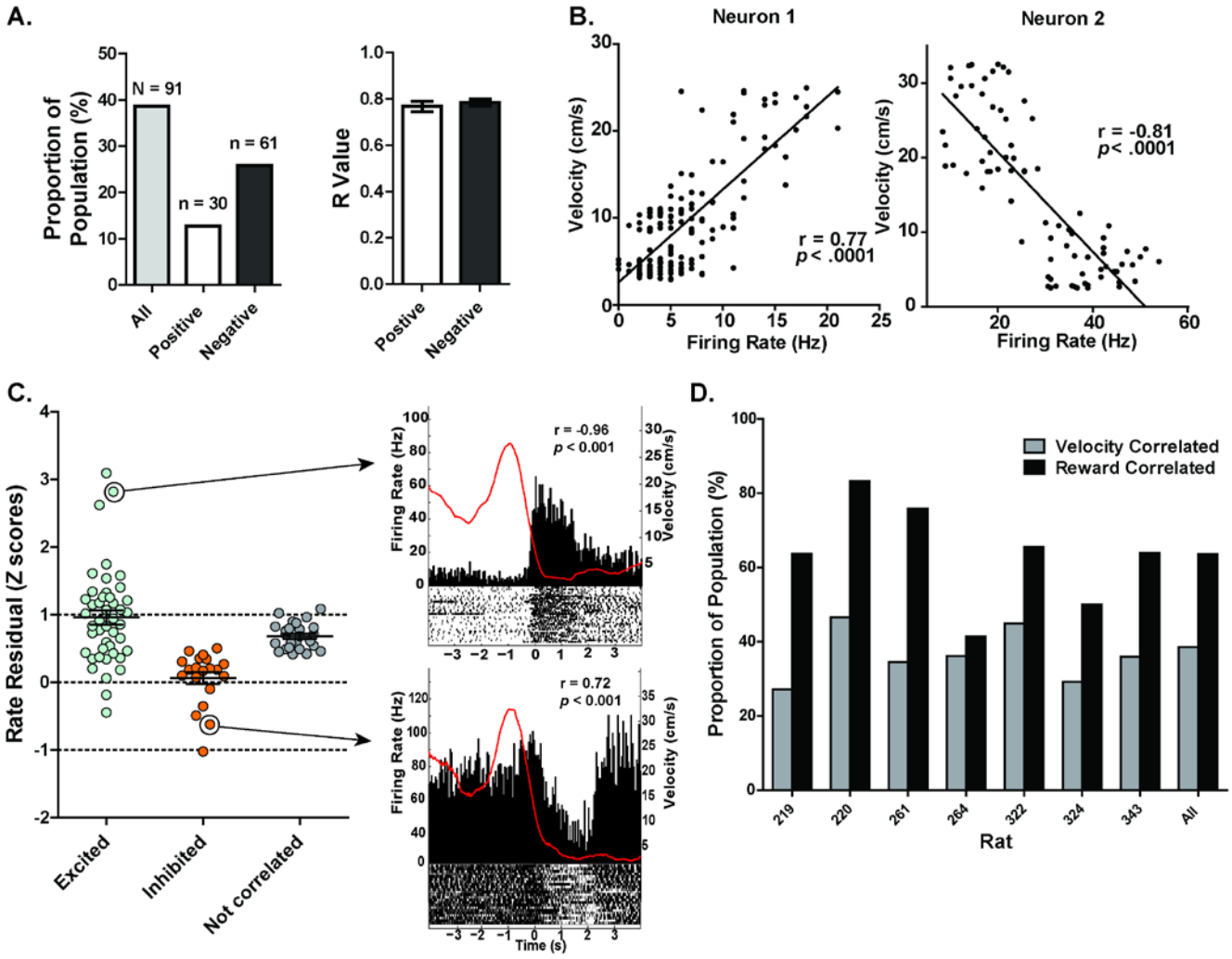



**Figure 3.5. A.** 38.40% of recorded PAG neurons were significantly correlated with velocity (Pearson correlation, $p \leq 0.05$). Of those, 12.66% were positively correlated while 25.73% were negatively correlated. The mean *R* values of positively- and negatively-correlated neurons were similar. **B.** Example of relationship between firing rate and velocity for a positively-correlated neuron (left) and negatively-correlated neuron (right). Y-axis represents velocity (cm/s) while the x-axis represents the corresponding firing rate (Hz) from the same time bin. **C.** Normalized firing rate residuals of velocity-correlated neurons from what would be predicted by velocity during the period when an animal encountered rewards. Neurons are separated into groups based on their relationships to rewards. We fit a linear equation of each neuron with velocity as the predictor during a window where the rats were not getting rewarded. Then, we calculated the difference between the expected firing rate and the observed firing rate (residual) during the period where the rat was getting rewarded. Residuals close to 0 indicate firing rate does not deviate from velocity-predicted values. The right panel represents 2 velocity correlated neurons that did not have residuals close to 0, suggesting that in these neurons there is conjunctive encoding of both movement and reward information. PETHs represent each neuron's responses to large rewards. Left Y axis represent the mean firing rate (Hz) over all events; right Y axis is mean velocity

(cm/s), represented by the red line. Time 0 indicates onset of large reward encounters. *p* and *r* values represent that neuron's relationship to velocity. **D.** Proportion of the population of recorded neurons that are correlated to velocity or reward separated by rat. Note that a neuron could be represented in either group. All rats had more reward correlated than velocity correlated neurons, although the exact proportion did vary from rat to rat.

The different columns of the PAG have been functionally separated based on their distinct contributions to analgesia, defensive behaviors, and autonomic regulation (Bandler and Shipley, 1994; Carrive, 1993). Thus, it stands to reason that the different columns of the PAG may process reward encounters differently. To investigate this, all recorded neurons were separated into different columnar groups: dorsomedial, dorsolateral, lateral, and ventrolateral (**Fig. 3.6,** see also **Fig. 3.3A.**). Unfortunately, not all of the columns were sampled equally and the majority of the neurons were recorded from the lateral (144/237; 60.76%) and dorsolateral columns (82/237; 34.6%) (**Fig. 3.6A**). Nevertheless, we were still interested in whether the reward-responsive neuronal subgroups would be proportionally divided within each column or if certain columns would show a distinct preference to respond differently to rewarding stimuli. Interestingly, we found that the lateral column had more reward excited neurons ($\chi^2$ (2) = 10.56; $p$ = 0.005) and the dorsolateral column had more non-reward correlated neurons ($\chi^2$ (2) = 13.86; $p$ = 0.001) than expected based on the responses of all neurons recorded (Fig 6B). These data suggest that there could be a functional subdivision of reward responsiveness by column in the PAG, as there are with other functions such as defensive behavior. Importantly, not all PAG neurons within a column responded the same as there was still heterogeneity of responses within each column (**Fig. 3.6C-F**).

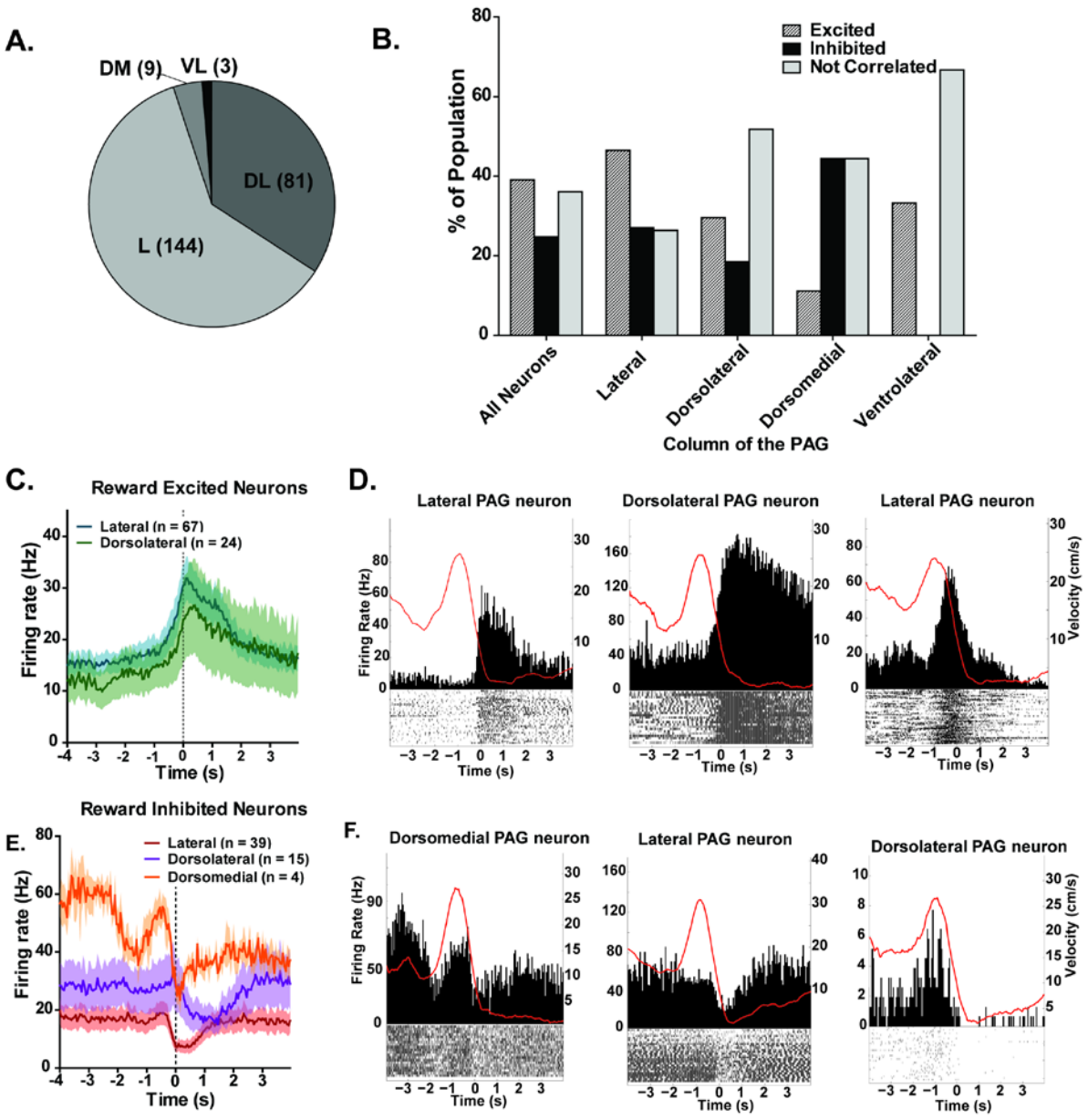



**Figure 3.6. A.** Number of PAG neurons recorded from each column of the PAG. The majority of neurons were recorded from the lateral and dorsolateral column. L= lateral, DL = dorsolateral, DM = dorsomedial, VL = ventrolateral. **B.** Proportion of population of neurons from each column that were reward excited, reward inhibited, or not correlated to reward. The lateral column had more reward excited neurons ($\chi^2$ (2) = 10.56; $p$ = 0.005) and the dorsolateral column had more non-reward correlated neurons ($\chi^2$ (2) = 13.86; $p$ = 0.001) than expected based on the responses of all neurons recorded. **C.** Mean firing rates of reward excited neurons in response to large rewards from the lateral and dorsolateral column. The ventrolateral and dorsomedial column were not represented here as there was only 1 or 2 neurons from each column. Time 0 indicates onset of reward encounters. The solid line indicates mean firing rate within 50ms time bins; the shaded region around each line represents ±SEM. **D.** PETHs of reward-excited neuron's responses to large rewards from the lateral (left), dorsolateral (middle), and lateral column

(right). Left Y axis represent the mean firing rate (Hz) over all events; right Y axis is mean velocity (cm/s), represented by the red line. Time 0 indicates onset of large reward encounters. **E.** Mean firing rates of reward inhibited neurons in response to large rewards by column. Time 0 indicates onset of reward encounters for large rewards. The solid line indicates mean firing rate within 50ms time bins; the shaded region around each line represents ±SEM. **F.** PETHs of reward-inhibited neuron's responses to large rewards from the dorsomedial (left), lateral (middle), and dorsolateral column (right). Left Y axis represent the mean firing rate (Hz) over all events; right Y axis is mean velocity (cm/s), represented by the red line. Time 0 indicates onset of large reward encounters.

**Discussion**

While a vast literature supports the view that PAG mediates the processing of fear and pain information, the present study provides the first clear evidence that PAG is also important for processing appetitive information, such as palatable rewards. We found that temporary and reversible inactivation of the PAG resulted in reduced reward consumption, decreased preference for large rewards, and more errors on a working memory task. An additional inactivation study in a separate group of animals showed that the PAG is need for normal food consumption in the absence of working memory demands as evidenced by decreased consumption of as well as increased latency to consume food. This result was not simply due to motor deficits as general locomotion was generally increased. Finally, we recorded PAG neurons in awake behaving animals as they performed a working memory task to collect differential sized rewards. We found bidirectional encoding of reward encounters in a subset of PAG neurons, with some neurons exhibiting significantly increased firing rates during reward encounters while the other subset exhibiting significantly decreased firing.

The anatomical evidence supports the view that the PAG encodes information that is relevant to basic behaviors of the animal, such as sexual, pain and threat-avoiding, as well as food-seeking and consumption. Further, the Motivation-Decision model (Fields, 2007) would predict that the PAG also serves to encode reward-relevant information when weighing behaviorally relevant stimuli to select the most appropriate action for a given situation. Indeed, we know that other brain regions canonically thought to be involved in defensive behaviors and threat detection, such as the amygdala and PBN, are also important for processing information relating to reward and food consumption. For example, the amygdala is important for reward and taste perception (Fontanini et al., 2009) and the PBN is essential for sensing gustatory stimuli (Scott and Small,

2009). Furthermore, both amygdala and PBN have reciprocal connections with the PAG, traditionally thought to be an important circuit for threat detection, anxiety, and fear (Rizvi et al., 1991; Krukoff et al., 1993; Krout et al., 1998). Together, these circuits may function to integrate other forms of salient information for adaptive action selection.

While the PAG projects to and receives projections from neurons in the VTA and amygdala, regions that show reward value prediction (Gottfried et al., 2003; Tobler et al., 2005), our current study does not support a role for PAG neurons in the encoding information that predicts reward value. PAG neurons' reward responses did not show representations of expectation of reward size, as reflected in the reward switch and omission data. This is in contrast to other valuation systems in the brain, such as dopamine neurons, which show increased firing in response to rewards that are larger than expected as well as the inverse for smaller than expected rewards. Like the PPTg (Norton, et al., 2011), another region that provides substantial excitatory input to VTA dopamine neurons, the PAG does not appear to be essential for determining prediction error codes.

A substantial subset (38%) of recorded PAG neurons are correlated with movement. It has been shown previously that many midbrain and hindbrain nuclei encode movement of the animal, i.e. PPTg, LDTg, VTA (Norton et al., 2011; Redila et al., 2015, Puryear et al., 2010). However, temporary inactivation of PAG did not cause motor impairment. Therefore, similar to suggestions made to account for strong velocity coding by VTA, LDTg, and PPTg neurons, PAG movement correlates may serve to keep track of ongoing behaviors to better prepare for future reward encounters. Indeed, in humans, it has been found that PAG neural activity increases when a threat is imminent, possibly in preparation for eliciting a predetermined flight motor pattern (Mobbs et al., 2007).

The food intake tests revealed that temporary inactivation of the PAG was sufficient to reduce food consumption in hungry animals. The PAG is an integral part of the endogenous opioid-mediated analgesia system and it is well known that opioids are important regulators of some aspects of food intake (Glass et al., 1999; Hagan et al., 2001; Levine and Billington, 2004). Systemic opioid agonist administration can increase food intake while antagonists decrease food intake (Cleary et al., 1996). It is thought that endogenous opioids function to regulate the hedonic aspects food intake via signaling at opioid-receptor dense striatal sites, especially the nucleus accumbens (Peciña and Berridge, 2000; Will et al., 2003; Kelley et al., 2005). Other areas important for opioid signaling in regards to food intake include the hypothalamus (Kelley et al., 2005), amygdala (Parker et al., 2014), PBN (Nicklous and Simansky, 2003), NTS (Giraudo et al., 1998), and VTA (Bodnar, 2004; Bodnar et al., 2005), regions known to be anatomically linked to the PAG. While opioid signaling in the PAG negatively regulates food intake (Jenck et al., 1987), the PAG may play an important role in regulating the hedonic value of food signaled elsewhere. Indeed, the reinforcing effects of systemic exogenous opiates such as heroin can be suppressed by intra-PAG infusions of naltrexone (Corrigall and Vaccarino, 1988) and infusion of morphine directly into the PAG is sufficient to induce conditioned place preference (Olmstead and Franklin, 1997; Le Merrer et al., 2009) suggesting that the PAG may be a necessary part of the opioid reinforcement circuit. There is already evidence that opioid-mediated pain circuits, of which PAG is major component of, are activated during appetitive circumstances (Fields, 2004). For example, chronic sucrose intake potentiates opioid mediated analgesia in the rat (Kanarek et al., 2001). Indeed, sucrose administration was sometimes used as an effective substitute for analgesia in infants undergoing minor but painful procedures (Bucher et al., 1995; Stevens et al., 2004). Even reward-associate cues can engage pain relief circuits. For

example, a study in rats found that their withdrawal threshold to a painful stimulus was increased in an environment where they previously received a palatable food reward, an effect that was reversed with systemic administration of naloxone (Dum and Hertz, 1984). Perhaps the reward responses the PAG neurons display in the current study are not responding to rewarding stimuli per se, but instead the neurons are activated in order to engage the descending pain modulatory circuit to favor approach behaviors to appetitive stimuli, especially in such cases that an animal is experiencing minor noxious stimuli. Thus, it is not surprising that inactivation of the PAG on the two tasks described here led to diminished food intake and increased latency to consume rewards as the circuit favoring ingestive behaviors was compromised. Alternatively, PAG inactivation could have affected neural areas that control orofacial movements or may have increased anxiety, which would have to be further explored.

Previous research has shown that the different columns of the PAG can be functionally divided because of their distinctly different contributions to autonomic processing, analgesia, and defensive behaviors (Bandler and Shipley, 1994). The columns of the PAG can also be anatomically divided based on distinct projection populations and neurotransmitter expression (Carrive and Bandler, 1991; Onstott et al., 1993; Carrive and Morgan, 2011). The current study found that reward processing may also be functionally divided by column. We found that proportionally more lateral PAG neurons displayed excitation in response to reward encounters whereas proportionally more dorsolateral PAG neurons were not correlated to reward at all. If the reward-correlates are indeed a reflection of the opioid-mediated endogenous pain relief system becoming engaged, this would make sense in light of the fact that the dorsolateral column of the PAG is not involved in opioid-mediated analgesia (Morgan, 1991).

We present data that provides novel evidence that the PAG may play a larger role in appetitive behaviors than previously shown. However, a limitation of the current study is that the pharmacological inactivation of PAG neurons was not selective to any particular neuron type or projection subpopulation. Additionally, the recording study does not tell us what type of neurons are responding to reward and which are not. The data do show that there is not a time delay in responding to rewards between the reward inhibited and reward excited subpopulations. This implies that one population is not directly influencing the other, but that they are responding at similar times to rewarding stimuli. However, it is important to remember that within each subpopulation, there was a heterogeneity in the timing of responses to reward stimuli. Thus, it is possible that the groups of neurons recorded were exerting control over the responses of other types of neurons. The current study reveals that the PAG is an important region for processing rewarding stimuli and ingestive behaviors, and this previously unstudied role should be further examined to gain insight into how neural systems interact to select the most adaptive behavior when competing motivations and stimuli are present. Understanding how neural systems that mediate threat detection and reward processing may interact and integrate for adaptive behavior selection will aide in understanding what is occurring in the brain when these systems have gone awry, such as in cases on anhedonia or anxiety disorders (Dillon et al., 2013).

## Chapter 4: Age-related changes in risk-based decision-making

### *Introduction*

According to the world health organization (WHO), in almost every country, the proportion of people aged over 60 years is growing faster than any other age group as a result of both longer life expectancy and declining fertility rates. An accompaniment of aging is changes in behavior, which is governed by underlying changes in brain physiology. Understanding how the brain normally ages and affects behavior will allow us to adapt to the new population landscape. Normal cognitive aging does not usually result in great losses in the number of neurons but is often accompanied by changes in decision making and memory functions even in the absence of neurodegenerative disease (Morrison and Hof, 1997; Fletcher & Rapp, 2013; Rapp and Gallagher, 1996; Rapp et al., 2002). Thus, an important avenue of current research is to understand how behavior changes with age, and the mechanisms mediating brain aging. However, studying brain aging in humans can be difficult as their life-expectancy is many decades and the invasive techniques needed to probe brain aging are not appropriate for use in humans. On the other hand, rodents are an excellent model to investigate age related changes in brain and behavior. They do not naturally develop neurodegenerative diseases like Alzheimer's and Parkinson's disorders. They have a relatively short lifespan for a mammal (~2-3 years) which enables researchers to study aged animals on a reasonable timeline. But most importantly, research shows that rodents and humans show similar behavioral and cognitive changes in old age (Gallagher et al., 2011).

Neurocognitive processes that are mediated by the hippocampus, such as declarative memory and pattern separation, and the dorsolateral prefrontal cortex (PFC), such as cognitive flexibility and working memory, are the most susceptible to age-related changes (Morrison & Baxter, 2012;

Barense et al., 2002; Dumitriu et al., 2010). It is thought that decline in separate neural systems occurs independently from each other; thus, declines in PFC function does not necessarily imply deficits in hippocampal function and vice versa (Fletcher & Rapp, 2013; Gallagher, et. al., 2011; Moore, et. al., 2009). We also know that cost-benefit decision making is altered with age, albeit to what extent is not known. In humans, it was found that older individuals tend to choose sure gains and avoid sure losses (Mather et al., 2012), avoid risky choices (Lee et al., 2008), and that gains and losses are encoded asymmetrically by older individuals (Samanez-Larkin et al., 2007). Aging has also been associated with an increase in risky decisions in some rats and humans and risk-aversion in others (Gilbert et al., 2012; Mata et al., 2012; Samson et al., 2015). The findings of individual differences in probability discounting in aged rats are consistent with evidence for individual differences in aged rat, monkey, and human performance in other cognitive domains (Gallagher et al., 1993, 2011; Barense et al., 2002; Schoenbaum et al., 2006; Bizon et al., 2009; Morrison & Baxter, 2012). On average, however, older individuals (animals and humans) tend to display more risk-aversion during risk-based decision making. Therefore, the goal of the current study was to investigate in more detail how normal aging affects risk-based decision making. To do this, we used a probability discounting task that tested rats' preference for a lever that led to a large reward with varying probability (the risky option) or a lever that lead to a small reward 100% of the time (the certain option). The probabilities associated with the risky lever were 100%, 50%, 25%, and 12.5%. The central question was whether aged rats show a preference for the small, certain reward over the risky one, even when the outcome would be better if the risky option was selected. Like aged humans tested on similar tasks, we expected aged rats to display significantly increased discounting behavior compared to young controls.

Also of interest was the question of how aging may influence risk-based decision making because we know that normal aging does not affect all brain systems equally as some systems are more prone to functional decline over time. For example, dopamine function declines across the life-span, and this decline has been correlated with age-related cognitive deficits (Backman et al., 2006; Rollo, 2009). Midbrain dopamine (DA) neurons of the ventral tegmental area (VTA) appear highly evolved to have strong responses to rewards and biologically salient events. They exhibit burst-like activity in response to primary rewards such as food and water (Wise, 2004; Fields et al., 2007). It is thought that these specialized responses to salient and rewarding events are important for learning and selection of appropriate behavioral responses. Therefore, DA functional decline over the lifespan could alter cognition reliant on DA. Indeed, evidence suggests that an age-related decrease in DA release is linked to poorer working memory function and perceptual speed (Backman et al., 2010). Additionally, DA hypofunction has been implicated in decreased sensitivity to changes in reward magnitudes (Dreher et al., 2008). However, it is not known if functional decline in DA function is linked to altered risk based decision making observed in old age. We know that midbrain DA neurons encode the uncertainty of probabilistic rewards (Fiorillo et al., 2003) and that alteration of DA signaling alters risky choice (St. Onge and Floresco, 2009; St. Onge et al., 2010). Therefore, another goal of the current project was to investigate how reward processing may change in the aged brain by recording neural activity from DA neurons of the VTA.

### *Materials and Methods*

*Subjects*. Fifteen 6-9 month and nineteen 25-26 month old male Long-Evans rats were received from the Laboratory of Behavioral Neuroscience at the National Institute on Aging, National Institutes of Health (Baltimore, MD). All rats are originally from the Charles River facility

(Raleigh NC). Twenty-eight rats (12 young and 16 aged) were used in the behavioral experiments and 6 rats (3 young and 3 aged) were used for the recording experiment. Upon arrival, rats were housed individually in Plexiglas cages and were maintained on a 12 h light/dark cycle (lights on at 7:00 A.M.). All behavioral experiments were performed during the light phase of the cycle. Each rat was allowed access to water ad libitum and food-deprived to 80-85% of its ad libitum feeding weight. In some cases of extreme obesity, rats were further restricted to 75% of their free-feeding weight with oversight from veterinary staff. Rats were handled and weighed daily. Rats that developed any health concerns were evaluated by veterinary staff and excluded from the experiment immediately if appropriate. All animal care and use were conducted in accordance with University of Washington's Institutional Animal Care and Use Committee guidelines.

*Water maze testing.* Standard water maze training (for detailed description, see Gallagher et al., 1993) was conducted for all rats in both experiments. Briefly, training took place across 8 consecutive days, three trials per day (each using a 90 s cutoff), with a 60 s intertrial interval. Every sixth trial was a probe test in which the escape platform was initially retracted to the bottom of the maze for 30 s and then made accessible to permit escape. Performance was evaluated according to learning index scores, as described previously (Gallagher et al., 1993). Briefly, the learning index scores were calculated as the weighted average proximity (in centimeters) to the hidden escape location across probe trials. This measure is optimized for identifying reliable individual differences in memory, and was used to classify aged animals as either aged unimpaired (AU), or aged impaired (AI), using criteria validated in earlier research (Gallagher et al., 1993). By this measure, lower values reflect closer proximity to the escape location and better spatial learning. The day after completing the spatial protocol, rats were

tested on a one-session, hippocampus-independent cued version of the Morris water maze. Six trials were given from multiple start locations, 60 sec maximum trial length.

*Probability-discounting task*

*Apparatus.* All behavioral testing for both experiments took place in Med PC operant chambers (30.5 × 24 × 21 cm; Med-Associates, St Albans, VT). Operant chambers were enclosed in sound-attenuating boxes with a fan to provide ventilation. While standard chambers are equipped with a metal grid floor, custom cut Plexiglas covered the floors for all rats to provide aged animals with a comfortable standing surface. The chamber was illuminated by a houselight on one side of the chamber. On the opposite wall from the house light was a food cup in the center of one wall and two retractable metal levers on either side of the food cup. A single light was located above each lever. For recording experiments, the food receptacle was custom built to extend from the wall to allow implanted animals to easily obtain the food reward. Additionally, the metal levers were coated in plastic to dampen electrical noise during neural recording experiments. Food rewards were 45 mg sugar pellets (Bioserv) that were dispensed from a pellet dispenser. Nose-pokes were recorded via two infrared photobeams on either side of the food cup.

*Pre-Training.* Animals underwent identical pre-training procedures for both experiments. Training protocols were adapted from St Onge and Floresco (2009). Once food restricted to 80-85% of their free-feeding weight, rats were given sugar pellets in their home cage to avoid neophobia for 1 to 2 days prior to training in the operant chamber. Then, rats were randomly assigned to one of the operant chambers that would then stay consistent throughout training. The first day of pretraining consisted of lever-press training at a fixed-ratio 1 schedule. Crushed sugar pellets were placed on the lever to encourage the rat to press the lever until the rat pressed started

pressing reliably. Sugar pellets were also placed in the food cup. Rats were trained on the FR1 schedule of lever pressing for a single lever until they reached the criteria of 60 lever presses within 30 minutes after which they switched to the same training protocol on the other lever. The first lever presentation was counterbalanced between rats. Then, rats trained on a simplified version of the probability discounting task. This task consisted of 90 trials during which 1 of the 2 levers was extended with the house light illuminated (left and right lever was presented once for every two trials and the order of presentation was randomized within a pair of trials). A lever press during one of these trials resulted in the delivery of a sugar pellet with a 50% probability. Once pressed, the lever retracted, the house light turned off and another trial was initiated after an inter-trial interval. If the rat failed to pressed the lever within 10 seconds, the lever retracted, the house light turned off, and the trial was recorded as an omission. Every trial was 40 seconds long regardless of the behavior of the animal. This procedure was used to allow the rats to learn about the probabilistic nature of lever pressing. Rats were trained on this version of the task until they omitted 10 or less trials per session. Days to reach criterion for each task varied from rat to rat (see results).

*Probability-discounting task: Behavioral experiments.* The task was based on previous studies investigating probability discounting (Cardinal and Howes, 2005; St Onge and Floresco 2009). After pretraining, rats performed 48 minute sessions consisting of 72 trials 5 days a week. Each session was separated into 4 different probability blocks of 18 trials. A trial began every 40 s with the illumination of the houselight and, 3 s later, insertion of one or both levers into the chamber. One lever was designated the Large/Risky lever, the other the Small/Certain lever, which remained consistent throughout training (counterbalanced left/right). Half of the rats were assigned their preferred lever as the risky lever and the other half were assigned their non-

preferred lever as risky. If the rat did not respond by pressing a lever within 10 s of lever presentation, the lights turned off, the levers were retracted, and the trial was scored as an omission. When a lever was chosen, both levers retracted. Pressing the certain lever led to the delivery of 1 sugar pellet 100% of the time. Pressing the 'risky' lever led to 4 sugar pellets with varying probability across trial blocks. The probability blocks were presented in descending order. The first block was associated with a 100% probability of receiving the large reward when the risky lever was pressed, then 50%, then 25%, and finally 12.5%. When food was delivered, the houselight remained on for another 4 s after a response was made, after which the chamber reverted to the intertrial state (darkness). Multiple pellets were delivered 0.5 s apart. The 4 probability blocks were separated into 8 forced-choice trials where only one lever was presented (4 trials for each lever, randomized in pairs) allowing rats to learn the amount of food associated with each lever press and the respective probability of receiving a reward over each block. This was followed by 10 free-choice trials, where both levers were presented and the rat chose either the Small/Certain or the Large/Risky lever. For each session and trial block, the probability of receiving the risky reward was drawn from a set probability distribution. Using these probabilities, selection of the Large/Risky lever would be advantageous in the first 2 blocks, and disadvantageous in the last block, whereas rats could obtain an equivalent number of food pellets after responding on either lever during the 25% block.

*Probability-discounting task: Recording experiments.* A modified version of the probability discounting task was used for rats in the recording experiment to facilitate training of the old rats. Rats were trained on a probability discounting task until behavioral criterion were reached using only two probability blocks: 80% and 20%. Each probability block consisted of 10 forced

choice trials and 20 free choice trials. Behavioral criterion was defined as stable behavior over 5 days (two-way ANOVA: no effect for day, significant effect of probability).

*Electrode Preparation and Surgical procedures*. Recording tetrodes were constructed from 20 µm lacquer-coated tungsten wires (California Fine Wire) and mounted on an array of two independently adjustable in-house made microdrives (3 tetrodes per microdrive). Tetrode tips were gold-plated to reduce impedance to 0.1– 0.4 MΩ (tested at 1 kHz). To implant recording electrodes, each rat was placed in an induction chamber and deeply anesthetized under isoflurane (4% mix with oxygen at a flow rate of 1 L/min). Under deep anesthesia, the animal was placed in a stereotaxic instrument (David Kopf Instruments) and anesthesia was maintained throughout surgery by isoflurane (1-2.5%) delivered via a nosecone. The skull was exposed and adjusted to place bregma and lambda on the same horizontal plane. After small burr holes were drilled, the microdrives were unilaterally implanted into the VTA (Young: -6.0 mm posterior, 0.5 mm lateral, and 7.0 mm ventral to bregma. Aged: -7.5 mm posterior, 0.5 mm lateral, and 7.0 mm ventral to bregma). Different coordinates for young and aged animals were used as previous experiments showed that the coordintates in aged animals do not follow those in a standard brain atlas. To address this, a small subset of aged rats underwent a lesioning procedure to obtain the optimal coordinates for targeting the VTA. Microdrive arrays were secured in place with anchoring screws and dental cement. Rats recovered for 7 days, during which they were weighed and handled daily.

*Single-unit recording and postsurgical procedures.* After a week of recovery, rats were returned to a food-restricted diet and spontaneous neural activity in the VTA was monitored as follows: the electronic interface board (Neuralynx, Bozeman, MT) of the microdrives was connected to

preamplifiers, and the outputs were transferred to a Cheetah data acquisition system (Neuralynx). Signals were filtered between 0.6 and 6 kHz, and digitized at 16 kHz. Acquired waveforms were not inverted. Neuronal spikes were recorded for 2 ms after the voltage deflection exceeded a predetermined threshold at 500 –7000X amplification. If no units were encountered, tetrodes were lowered in 40 µm increments to target new units. A non-motorized commutator (SwivElectra, Crist Instrument Co., Hagerstown, MD) with custom Neuralynx adapters was mounted above the operant chamber and used to prevent the tether from getting tangled. A video camera mounted on the ceiling of the chamber recorded video and data were relayed to the acquisition system. Once clearly isolated and stable units were found, the probability discounting task and recording began. Daily recording sessions occurred when rats performed in an operant box that was embedded within a sound attenuated chamber. Animals' behavior was recorded simultaneously with neural data, and time-stamps in the form of TTL pulses were instantaneously sent to the neural recording system via the SuperPort TTL card (DIG-726) and the Cheetah data acquisition system. The commands sent by Med PC to run the behavioral experiment were also recorded in the Neuralynx event file as TTL pulses via a SmartCtrl connection panel (SG-716B) (Zheng and Ycu, 2012). Experimental sessions continued until tetrodes passed through the VTA based on the distance traveled from the brain surface.

*Histology*. After the completion of all recording sessions, tetrode locations were verified. Rats were deeply anesthetized under 4% isoflurane. The final position of each tetrode was marked by passing a 15A current through a subset of the tetrode tips for 15 s. Then, the animals were given an overdose of sodium pentobarbital and transcardially perfused with 0.9% saline and a 10% formaldehyde solution. Brains were stored in a 10% formalin–30% sucrose solution at 4°C for 72 h. The brains were frozen, and then cut in coronal sections (45µm) on a freezing sliding

microtome. The sections were then mounted on gelatin-coated slides, stained with cresyl violet, and examined under light microscopy. Only cells verified to be recorded in VTA were included in the data analysis.

*Data analysis*. Single units were isolated using an Offline Sorter (Plexon). Various waveform features, such as the relative peak, valley, width, and principle component, were compared across multiple units simultaneously recorded from the four wires of a tetrode. Only units showing good recording stability across the entire recording session were included. Further analysis of the sorted units was performed with custom Matlab software (Mathworks). To examine the reward-related responses of VTA neurons, peri-event time histograms (PETHs) were constructed at 4.0 s around the time of all reward acquisition-triggered events. A bin size of 100 ms was used for all PETHs. VTA cells were identified based on phasic responses to reward acquisition, lever cues, and pellet cues. A VTA neuron was considered reward or cue responsive if it doubled its firing rate during the 300 ms from cue or reward acquisition onset compared to its baseline firing rate. Putative DA and non-DA cells were classified using a cluster analysis developed to identify DA cells in the rodent VTA (Jo et al., 2013; Roesch et al., 2007; Jin and Costa, 2010). Detailed information of this analysis can be found in previous reports (Jo et al., 2013; Roesch et al., 2007; Takahashi et al., 2009, 2011). Briefly, using the tetrode channel with the largest peak-to-valley amplitude, two basic characteristics of the average spike waveform were determined for each cell: (1) the half time of the spike duration (i.e., measured between the first valley and the next peak); and (2) the amplitude ratio of the first positive peak and negative valley in a waveform [$(n - p)/(n + p)$, with $n$ as the first negative valley and p as the first positive peak]. A scatter plot including all VTA cells was then constructed for young and aged rats together, as well as for each age group separately. The cluster that included neurons with waveforms

showing a broad half duration and low amplitude ratio was putatively classified as DAergic. Neurons that fell into multiple clusters were not classified. Spontaneous firing properties of putative DA cells were calculated from data collected during the ITIs (i.e., when rats were not engaged in the task-related behavior).

*Statistical analysis.* Statistical analyses were performed using SPSS 22.0, Graphpad Prism 6.0, or custom Matlab software (Mathworks). The statistical tests used are indicated in the results section where appropriate.

***Results***

After stable behavior was reached, the mean choice of the large reward at each probability block was calculated for each rat. Each rat's performance was averaged over the five-day stable behavior period. Performance scores reflect rats' choice of the large reward lever during the choice blocks. The choice of the large reward was calculated only from those trials in which rats participated (i.e. and not omission trials). Then, rats' scores were grouped by age. A mixed factorial ANOVA was run to assess the effects of age and probability on choice behavior. There was a significant main effect of probability ($F$ (3,104) = 43.53, $p < 0.001$) and a significant main effect of age ($F$ (1,104) = 7.93, $p < 0.01$), with aged rats showing reduced choice of the large reward over the four probability blocks (**Figure 4.1A**). However, if mean choice of large reward is collapsed for all 4 probabilities, this effect is no longer significant ( $p > 0.05$ )(**Figure 4.1B, C**). When viewing each rat's individual discounting behavior, the trend for aged animals to choose the large risky reward less than their younger counterparts becomes more obvious, albeit there is some variability in the level of discounting within an age group (**Figure 4.1D, E**).

However, as a group, aged rats showed significantly increased probability discounting than young rats.

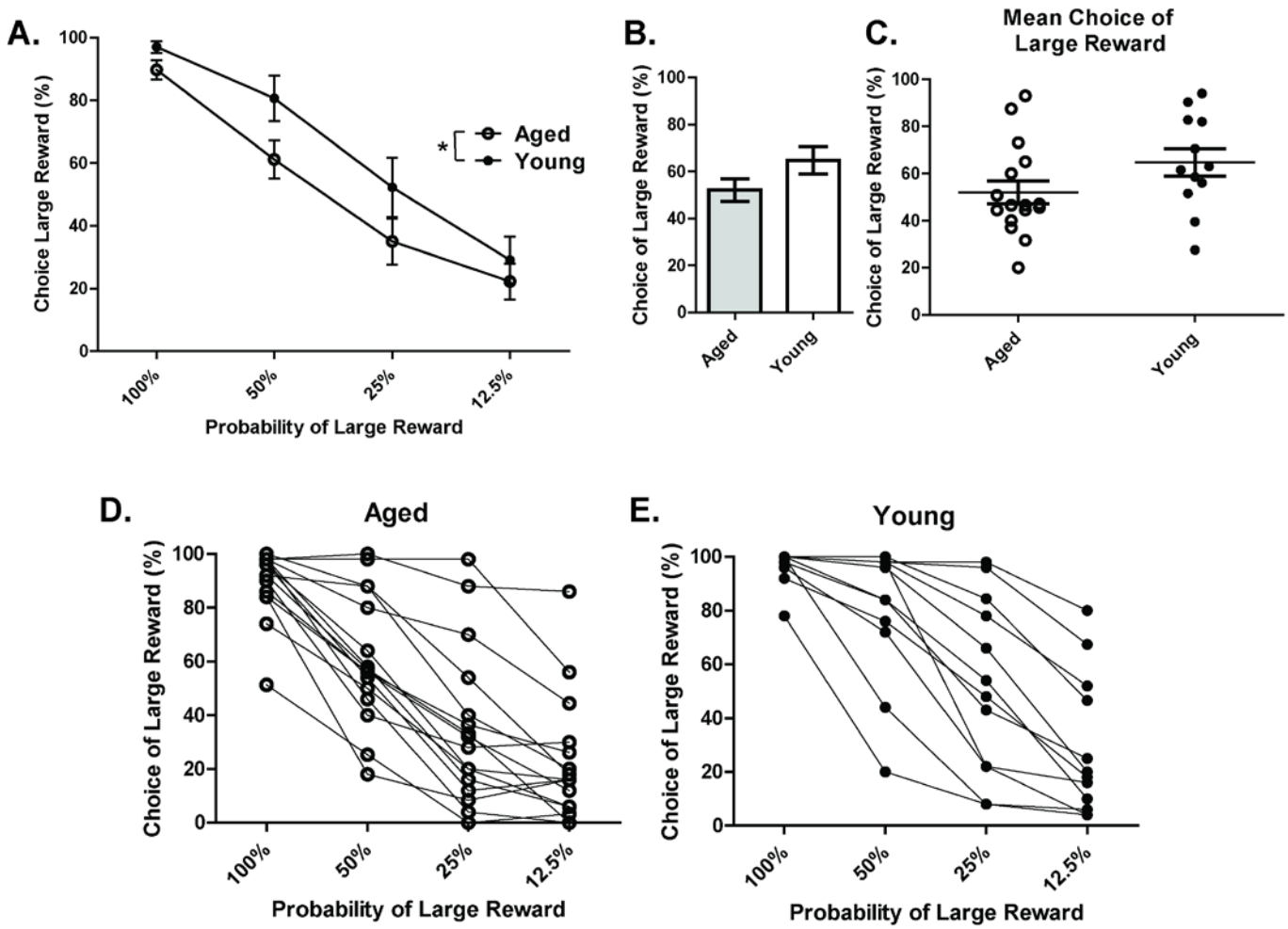



**Figure 4.1. A.** Mean choice of large reward for all probability blocks grouped by age. Scores reflect rats' choice of the large reward lever during the choice trials that rats participated (i.e. and not omission trials). Aged rats chose the large risky lever significantly less than young rats ($F(1,104) = 7.93$, $p < 0.01$). **B.** Mean choice of large reward by age group collapsed across all probability blocks. There was no significant effect of age (independent t test, $p > 0.05$). **C.** Mean choice of large reward by age group collapsed across all probability blocks for individual rat behavior. **D.** Individual aged rats' choice behavior across all four probability blocks. **E.** Individual young rats' choice behavior across all four probability blocks. Open circles: aged rats, n = 16; Closed circles: young rats, n = 12.

To gain more insight into how aged rat's choices led to increased discounting, we next conducted a win-stay, lose-shift analysis. The ability to make flexible decisions based on choice feedback is important for optimal decision making. However, being too sensitive to losses or not sensitive enough to positive feedback may reduce gains in probabilistic paradigms. Aged rats have previously been shown to have win-stay and lose-shift deficits (Means and Holsten, 1992). A win-stay deficit can be defined as not repeating a choice that was previously rewarded, even when it is beneficial to do so. A lose-shift deficit can be defined as not switching to the other option after a choice was not rewarded/correct. Win-stay and lose-shift behavior is thought to reflect sensitivity to positive and negative reinforcement, respectively. Thus, we wanted to assess if the increase in probability discounting observed with age related to distinct changes in sensitivity to positive or negative reinforcement. To do this, a win-stay and lose-shift choice ratio was calculated for each rat. For win-stay, the ratio reflected the number of choices for the risky lever after a risky win out of the total number of trials for which there was a risky gain. The ratio of lose-shift choices was calculated as the number of choices for the small certain lever after the risky lever was chosen and no reward was delivered, i.e. a loss, out of the total number of loss trials. These ratios were calculated for each rat, and rats were then grouped by age for analysis. It was found that aged rats had significantly reduced win stay choice ratios compared to young rats (independent t test, $t(24) = 2.42$, $p = 0.01$) (**Figure 4.2A**). However, when lose-shift choices were compared, aged and young rats displayed similar lose-shift behavior (independent t test, $t(24) = 0.69$, $p = 0.25$) (**Figure 4.2A**). This suggests that aged rats have a selective deficit in maintaining risky choices after positive reinforcement and are not necessarily more loss-averse than their younger counterparts.

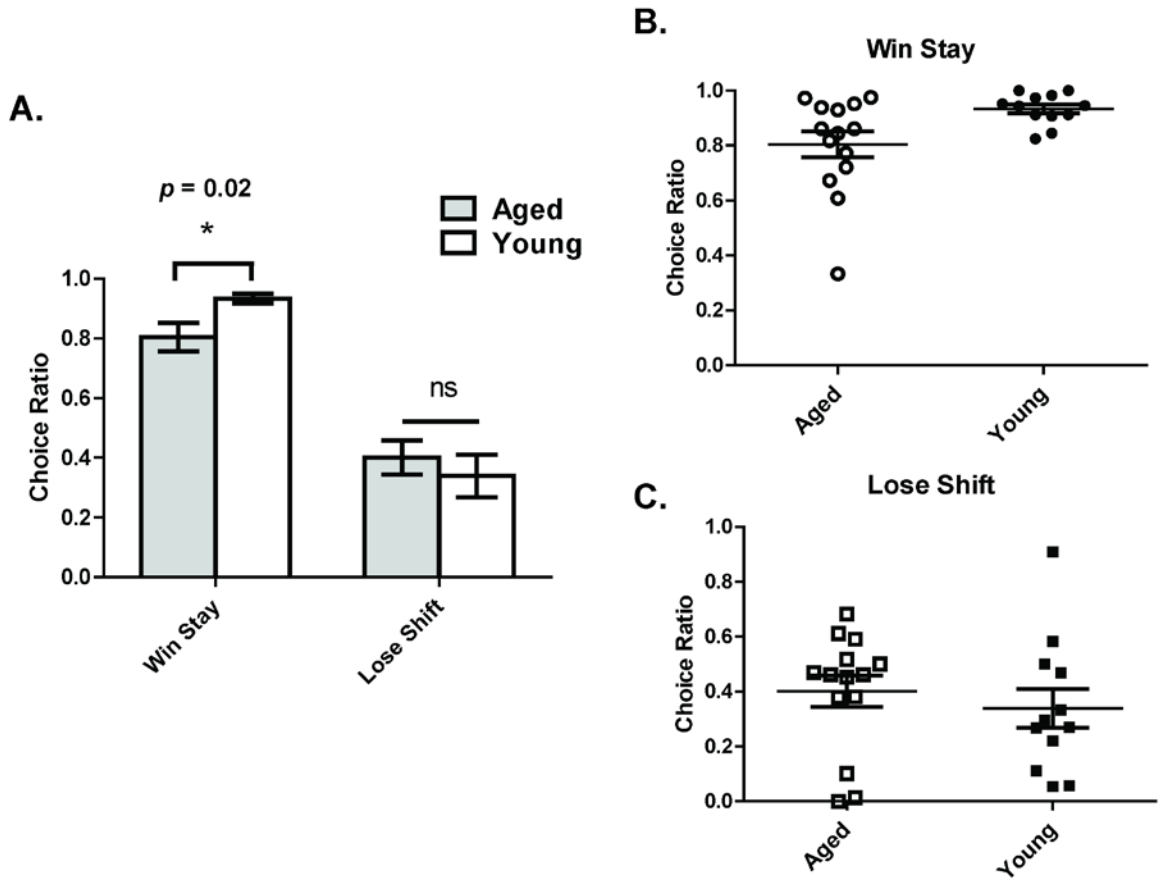



**Figure 4.2. A.** Win-stay and lose-shift choice behavior. The choice ratio for win-stay represents the ratio of choices that were for the risky lever following a "win" for all rewarded risky trials. A win is defined as a choice for the large/risky lever that resulted in a reward. Aged rats had significantly reduced win-stay ratios compared to young rats (independent t test, t (24) = 2.42, $p$ = 0.01). The choice ratio for lose-shift represents the ratio of choices for the small/certain lever after a "loss". A loss is defined as a choice for the large/risky lever that did not yield a reward. There was no difference in lose-shift behavior by age group (independent t test, t (24) = 0.69, $p$ = 0.25). **B.** Individual rat win-stay choice ratios. **C.** Individual rat lose-shift choice ratios. Open symbols: aged rats, n = 16; Closed symbols: young rats, n = 12.

We next assessed if age-related changes on risk based decision making was related to deficits in spatial memory. Previous research suggests that deficits on spatial memory tasks with age can be selective, indicating that age-related decline in the hippocampus happens independently of changes in other brain regions (Gilbert et al., 2012; Samson et al., 2015). To assesses this in the current study, individual choice behavior on the probability discounting task was correlated with performance on the water maze. A learning index score (LIS) was calculated based on performance on multiple probe trials on the water maze. LIS reflects the average proximity of the animal during probe trials to the training location of the escape platform; lower index scores indicate more accurate proximity and is used as an approximation of spatial memory (Gallagher et al., 1993). Rats are considered "impaired" with a LIS ≥250. Learning index scores have been shown to be associated with age-related changes in spatial memory (Gallagher et al., 1993, Nicolle et al., 1999). As expected, aged rats had significantly higher learning index scores compared to young rats, even when collapsing aged impaired and unimpaired groups (one-tailed independent t test, $t$ (26) = 5.23, $p < 0.0001$) (**Figure 4.3A**). When grouping all 28 rats together, no significant relationship between choice behavior on the probability discounting task and water maze performance was observed (Pearson correlation, $R$ = -0.14, $p$ = 0.47) (**Figure 4.3B**). We then compared choice behavior and water maze performance separately by age group. While not significant, there was a negative relationship between large reward choice behavior and LIS in young rats ($R$ = -0.55, $p$ = 0.06) (**Figure 4.3D**) but a positive relationship between large reward choice and LIS in aged rats ($R$ = 0.39, $p$ = 0.13) (**Figure 3C**), i.e. the more an aged rat chose the risky reward the more spatially impaired they were on the water maze task. However, these relationships were not statistically significant and therefore may not reflect a true relationship between these two behaviors.

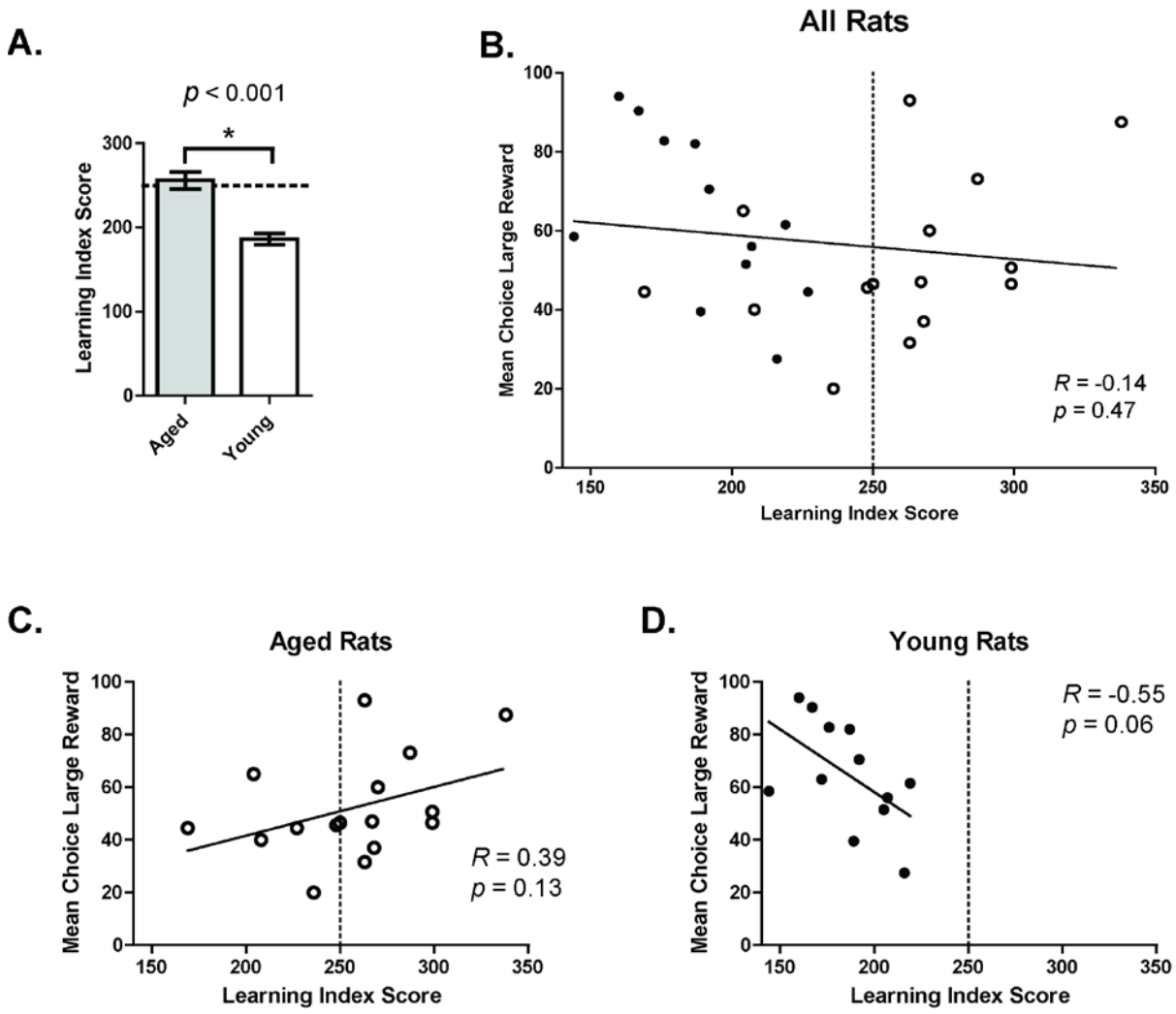



**Figure 4.3. A** Mean learning index score (LIS) by age group. Aged rats had significantly higher learning index scores compared to young rats, even when collapsing aged impaired and unimpaired groups (one-tailed independent t test, $t$ (26) = 5.23, $p < 0.0001$). **B.** Mean choice of large reward collapsed across all probability blocks on probability discounting task (%, Y axis) correlated to LIS on water maze (X axis). No significant relationship between choice behavior on the probability discounting task and water maze performance was observed (Pearson correlation, $R$ = -0.14, $p$ = 0.47). Dotted line represents when rats are considered impaired on the water maze. Open circles: aged rats, n = 16; Closed circles: young rats, n = 12. **C.** Relationship between risky choice and water maze performance for aged rats. No significant relationship was found between these two variables (Pearson correlation, $R$ = 0.39, $p$ = 0.13). **D.** Relationship between risky choice and water maze performance for young rats. No significant relationship was found between these two variables (Pearson correlation, $R$ = -0.55, $p$ = 0.06).

Another aim of the current study is to investigate VTA (both DA and non-DA neurons) neural responses to probabilistic rewards in aged and young animals. We did this on a modified version of the probability discounting task in which there were only two probability blocks (see methods). We did not find any significant differences in the behavior of young and aged rats on this modified version (**Figure 4.4**) which is most likely due to the simplified nature of the task and high variability in individual rats' behavior (**Figure 4.4A, B**). Nevertheless, how the neural computations that govern valuation change with age may give us insight into the age-related differences we saw in Experiment 1. Loss of the appropriate phasic response to rewards may cause aged rats to interpret the risky reward as less reinforcing and thus be less sensitive to positive reinforcement. This could be a possible mechanism that reduces the ratio of win-stay choice behavior in aged rats. We recorded 269 neurons from the VTA in 6 rats. Of those, 38 (14.1%) were identified as dopamine neurons based on previously established electrophysiological criteria (see methods; Jo et al., 2013; Roesch et al., 2007) (**Figure 4.5A**). Putative DA neuron numbers were similar to previous reports (Roesch et. al. 36/258; 13.9% and Jo et al. study 203/905; 22%). However, there were proportionally fewer DA neurons recorded from aged rats than young (Aged: 11 DA neurons out of 115 total (10.4%); Young: 26 DA neurons out of 154 total (16.9%)). To potentially account for this age-difference, it is important to note that DA neurons show functional changes with age. While less sensitive to neuronal loss than the nigrostriatal system, DA neurons of the VTA display functional decline as DA metabolites (DOPAC, HVA) decline with age in the rat VTA (Goudsmit et al., 1990). VTA DA neurons display axonal degeneration, a build-up of amyloid precursor protein and alpha synuclein, and loss of tyrosine hydroxylase (TH) despite no obvious neurodegeneration (Cruz-Muros et. al., 2007). In the monkey, it was shown that functional decline in the DA system leads

to a decrease in DA release in response to rewards and drugs of abuse as D-amphetamine stimulation of DA release was significantly diminished by middle- and old-age monkeys compared to young animals (Gerhardt et. al., 2002). Most importantly, age can affect the electrophysiological properties of recorded DA neurons. In a previous report, it was found that DA neurons from aged mouse substantia nigra showed lower spontaneous firing rates, impaired firing fidelity, narrower spike widths, and decreased L-type calcium currents (Branch et al., 2014). Therefore, using previously established methods to identify putative DA neurons in the VTA may lead to exclusion of DA neurons in the aged rat. Nevertheless, our initial analysis of DA cell responses focused on neurons identified as DAergic based on published criteria (e.g. Jo et al., 2013). Of interest were DA neuron responses to probabilistic cues that indicate availability of reward (lever cue), cues indicating when a choice resulted in a reward (pellet cue), and the receipt of the reward itself (first nosepoke after a reward was delivered). Based on previous research, we know that DA neurons encode the value of probabilistic rewards: neurons exhibit a lower firing rate to cues that are associated with a lower probability of receiving reward compared to cues that are associated with a high probability of receiving reward, and DA neurons respond more to a primary reward that was preceded by a cue associated with a low probability of receipt (Fiorillo et al., 2003). Therefore, we hypothesized that DA neurons would exhibit phasic responses to the lever cue, with a higher firing rate to the cue during the 80% block compared to the 20% block. Additionally, we expected higher phasic firing of DA neurons to the pellet cue, with a higher firing rate during the 20% block compared to the 80% block. In the young rats, DA neurons indeed exhibited phasic firing to the lever cue that was significantly greater than baseline firing rates (Repeated measures ANOVA, significant main effect of time, $F(2,25) = 10.92$, $p < 0.001$) and this phasic response was greater during the high probability block

(Bonferroni post hoc multiple comparisons test, $t = 2.32$, $p = 0.01$) (**Figure 4.5C**). When examining these same neurons responses to the pellet cue, we found that they exhibited significantly increased firing rate to the pellet cue compared to baseline (Repeated measures ANOVA, significant main effect of time, $F(2,25) = 10.49$, $p = 0.01$) (**Figure 4.5D**), but they did not show significantly increased firing rate to this cue during the 80% block compared to the 20% block (Bonferroni post hoc multiple comparisons test, $t = 0.77$, $p > 0.05$). The aged rats' neurons, by comparison, did not show any detectable responses to any of these cues (**Figure 4.5E,F**). Additionally, overall rate of the putative aged DA neurons (0.77±0.24 spikes/s) was significantly reduced compared to young rats ((3.25±0.82 spikes/s), ($t(35) = 2.24$, $p = 0.02$) (**Figure 4.5B**).

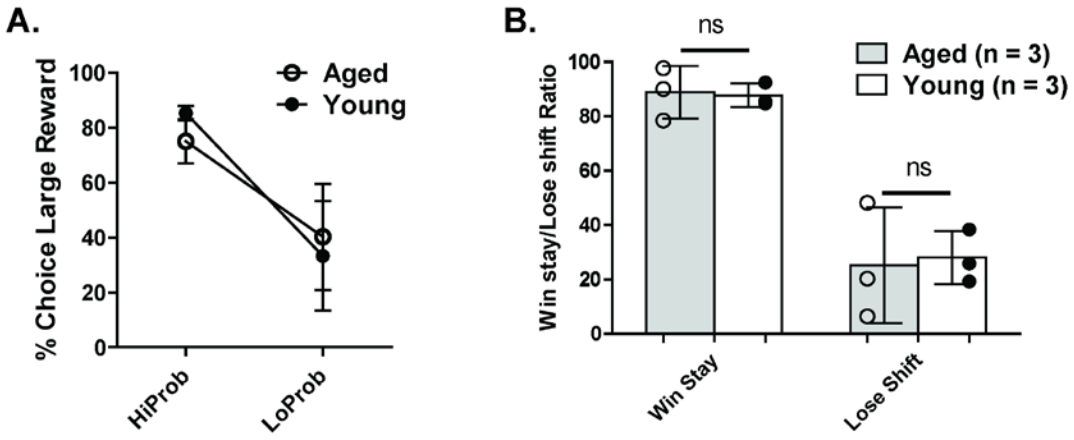



**Figure 4.4. A.** Percent choice of large reward by age group. HiProb = 80%; LoProb = 20%. **B.** Win-stay and lose-shift choice behavior. The choice ratio for win-stay represents the ratio of choices that were for the risky lever following a "win" for all rewarded risky trials. A win is defined as a choice for the large/risky lever that resulted in a reward. The choice ratio for lose-shift represents the ratio of choices for the small/certain lever after a "loss". A loss is defined as a choice for the large/risky lever that did not yield a reward. Open circles: aged rats, n = 3; Closed circles: young rats, n = 3.

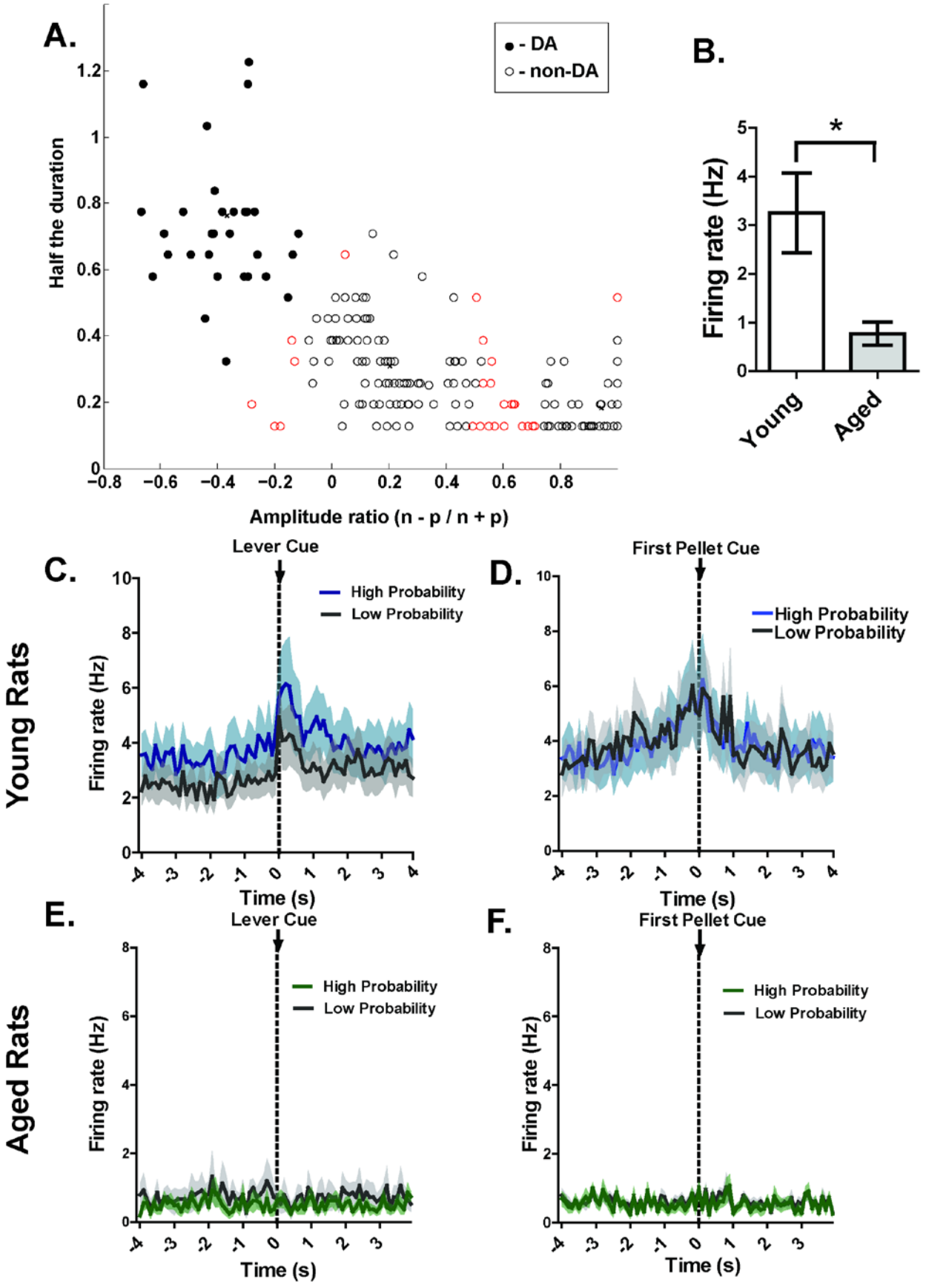

A.
● - DA
○ - non-DA
Half the duration
Amplitude ratio (n - p / n + p)
B.
Firing rate (Hz)
*
Young
Aged
Young Rats
C.
Lever Cue
High Probability
Low Probability
Firing rate (Hz)
Time (s)
D.
First Pellet Cue
High Probability
Low Probability
Time (s)
Aged Rats
E.
Lever Cue
High Probability
Low Probability
Firing rate (Hz)
Time (s)
F.
First Pellet Cue
High Probability
Low Probability
Firing rate (Hz)
Time (s)

**Figure 4.5. A.** Results of cluster analysis for all VTA neurons (N = 269). Putative dopamine (DA) cells were identified based on half spike duration and the amplitude ratio of the initial negative valley (n) and positive peak (p). Closed circles are putative DA neurons (n = 38); open circles are classified as non-DA neurons ( n = 231). **B.** Overall rate of the putative aged DA neurons (0.77±0.24 spikes/s) was significantly reduced compared to young rats ((3.25±0.82 spikes/s), ($t$ (35) = 2.24, $p$ = 0.02). **C.** Mean rate (Hz) of young rats' putative DA neurons (n = 26) centered around the lever cue (±4.0 sec). Blue traces indicate responses during the 80% block; gray traces represent the 20% block. DA neurons firing to the lever cue was significantly greater than baseline firing rates (Repeated measures ANOVA, significant main effect of time, $F$ (2,25) = 10.92, $p$ < 0.001) and this response was greater during the high probability block (Bonferroni post hoc multiple comparisons test, $t$ = 2.32, $p$ =0.01) **D.** Mean rate (Hz) of young rats' putative DA neurons centered around the first pellet cue (±4.0 sec). The neurons significantly increased firing rate to the pellet cue compared to baseline (Repeated measures ANOVA, significant main effect of time, $F$ (2,25) = 10.49, $p$ = 0.01), but they did not show significantly increased firing rate to this cue during the 80% block compared to the 20% block (Bonferroni post hoc multiple comparisons test, $t$ = 0.77, $p$ > 0.05).**E.** Mean rate (Hz) of aged rats' putative DA neurons (n = 11) centered around the lever cue (±4.0 sec). Green traces indicate responses during the 80% block; gray traces represent the 20% block **F.** Mean rate (Hz) of aged rats' putative DA neurons centered around the first pellet cue (±4.0 sec).

Since the DA waveform analysis could exclude DA neurons in the aged rats due to functional changes in the neurons over time, we could be neglecting neurons that do indeed encode the salient events of the task. Thus, the neural data were reanalyzed to include all VTA neurons for both age groups. Previous reports show that even non-DA neurons of the VTA encode salient events (Puryear et al., 2010), albeit with different firing patterns in response to reward-predicting cues (Cohen et al., 2012). In the present study, for the young rats, 37 neurons displayed a pattern of phasic excitation to the lever cue. The responses of these neurons during the first 300ms after the onset of the lever cue were compared between the high and low probability blocks to see if the phasic excitation of these neurons scaled with the probability of receiving the large reward. Indeed, the neurons displayed significantly greater firing rate responses to the lever cue during the high probability block compared to the low probability block (one-tailed dependent t test, $t$ (36) = 1.73, $p$ = 0.04). For the aged rats, only 9 neurons showed excitation to the lever cue and when these responses were analyzed to examine if they encoded the probability of available reward it was found that the neurons did not display a difference in firing rate between the two probability blocks (one-tailed dependent t test, $t$ (8) = 0.09, $p$ = 0.46). Therefore, the aged rats' VTA neurons did not distinguish between the cues that predict the probabilistic rewards with high and low probabilities while the young rats VTA neurons did. These results indicate that the increase in probability discounting in aged rats may be due to the blunting of the dynamic range of the VTA neurons' responses, which might prevent the aged rats from correctly assessing the value of the risky option.

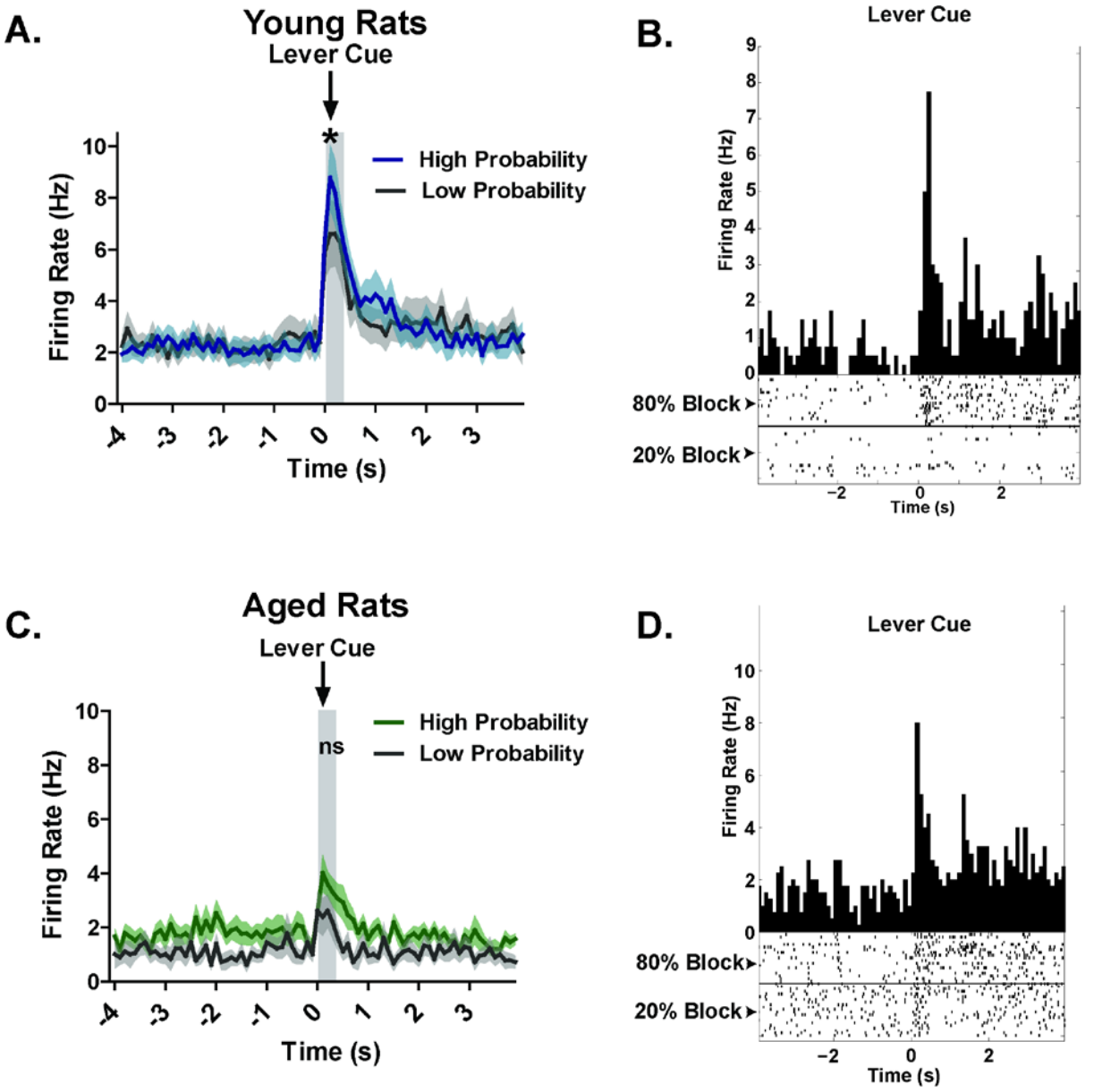



**Figure 4.6.** **A.** Mean rate (Hz) of young rats' lever excited VTA neurons (n = 37) centered around the lever cue (±4.0 sec). Blue traces indicate responses during the 80% block; gray traces represent the 20% block. The neurons displayed significantly greater firing rate responses to the lever cue during the high probability block compared to the low probability block (one-tailed dependent t test, $t$ (36) = 1.73, $p$ = 0.04). **B.** Peri-event time histograms (PETHs) of a lever excited neuron's responses to the lever cue during high and low probability blocks. Left Y axis represent the mean firing rate (Hz) over all events; time 0 indicates onset of lever cue. Raster diagrams show individual spikes around the lever cue. Top: 80% block; bottom: 20% block. **C.** Mean rate (Hz) of aged rats' lever excited VTA neurons (n = 9) centered around the lever cue (±4.0 sec). Green traces indicate responses during the 80% block; gray traces represent the 20% block. The neurons did not display a difference in firing rate between the two probability blocks

(one-tailed dependent t test, $t$ (8) = 0.09, $p$ = 0.46). **D.** Peri-event time histograms (PETHs) of a lever excited neuron's responses to the lever cue during high and low probability blocks. Left Y axis represent the mean firing rate (Hz) over all events; time 0 indicates onset of lever cue. Raster diagrams show individual spikes around the lever cue. Top: 80% block; bottom: 20% block.

***Discussion***

It is important to understand how and why risk-based decision making changes over the lifespan. The current data show that aged rats display decreased choice of the large risky reward, i.e. more discounting on the probability discounting task, reflecting an age-related change in risk-based decision making. Previous reports have not found this same age-related effect on probability discounting in rats (Gilbert et al., 2012; Samson et al., 2015). This apparent discrepancy could be due to rat strain differences or a difference in methodologies rather than a lack of an age-related effect on probability discounting. The findings here are in line with increased risk-aversion observed in older humans (Lee et al., 2008). Thus, we believe our model of risk-based decision making can be used to study the underlying physiological changes in the brain that contribute to the observed alterations in decision making with age.

We found that the increased discounting was related to alterations in choices after positive reinforcement as aged rats show decreased win-stay behavior compared to their younger counterparts. It has previously been shown that aged rats have a deficit in repeating a choice that was previously rewarded (Means and Holsten, 1992). On the other hand, we did not find a significant difference between the age groups in lose-shift behavior, indicating that aged animals are not necessarily more loss-averse than their younger counterparts. Therefore, age-related increases in discounting on the probability task are more likely to be due to changes in adequately assessing the positive outcomes of past choices. This effect seems to be selective for risky choices, as choice of the large/risky reward was not significantly different between aged and young rats when outcomes were certain, i.e. when the probability of the "risky" reward was 100% (independent t test, $t$ (26) = 1.84, $p$ = 0.08). Instead, deficits arose when risk was introduced, even when it is more beneficial to continue choosing the risky option, i.e. during the

50% block. This is in line with human research that shows older individuals selectively avoid risky options, even if they reduce total gains (Lee et al., 2008; Rutledge et al., 2016).

Age-related decline of hippocampal function has been vigorously studied in rats using spatial memory tasks like the Morris water maze. These tests reveal that aged rats consistently show impairment on hippocampal dependent tasks compared to young rats (Gallagher et al., 2011). Indeed, in vivo investigation into hippocampal function reveals that place cells are less likely to update in new environments, and this decrease in adaptability is correlated with deficits in spatial memory (Wilson et. al., 2004). Therefore, we were not surprised to find that the rats tested in this study showed significantly impaired water maze performance compared to younger animals. We were instead interested in whether the decline in spatial memory was correlated with altered risk based decision making. Although it is thought that cognitive decline with age in one brain region is not necessarily predictive of decline in other regions, most of this research is restricted to assessing the relationship between prefrontal cortex and hippocampal dependent tasks (Fletcher & Rapp, 2013; Gallagher, et. al., 2011; Moore, et. al., 2009). We found that choice behavior on the probability discounting task was not significantly related to water maze performance. This is consistent with previous similar studies that assessed whether there is a relationship between the two types of behavioral performances (Gilbert et al., 2012; Samson et al., 2015). Therefore, there is insufficient data at this time to link spatial working memory decline in age with increased probability discounting.

While the in-vivo electrophysiological data from young and aged rats' VTA are preliminary, the striking lack of dynamic range in VTA neurons of aged rats supports the view that a functional decline in these neurons could contribute to altered risk-based decision making. The reduced responsiveness of aged VTA neurons to rewarding stimuli may underlie the age-related reduced

sensitivity to positive reinforcement observed in the win-stay analysis. Further, the low rate and lack of responses by putative DA neurons of aged rats suggests that this effect could be compounded by functional decline of DA neurons. However, at this time, we cannot be confident that the neurons classified as dopaminergic in the aged rats are truly dopaminergic due to the possibility that the waveform characteristics have changed with age (Rollo, 2009; Branch et al., 2014). Future work is needed to characterize the identity of recorded VTA neurons in aged rats to elucidate if the blunted responses are due to changes in dopamine neuron function specifically or VTA neurons in general.

Normal aging is known to affect a wide range of cognitive abilities. The current data support previous research that showed that old age affects how decisions are made in risky situations. A useful model for examining risky decision making alteration late into the lifespan can help us understand how older individuals make decisions when faced with uncertain situations. Given the projected increases in the aged population across the world, understanding these changes has important implications for society. Additionally, understanding the physiological changes the brain undergoes with age can aide us in developing treatments to ameliorate any deleterious effects aging may have on cognition.

## Chapter 5: General Discussion

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The data presented in Chapter 1 lend new information about how memory systems encode information about values of past decisions by providing evidence that a proportion of hippocampal place cells update their spatially selective firing based on econometric variables, such as the value-based outcome of a decision. Chapter 2 outlines a novel role for a brain region previously regarded as mostly being important for pain processing and defensive behaviors. The data here do not contest these essential roles of the PAG, but instead lend new insight into how PAG neurons may also play a significant part in a circuit that shapes adaptive behaviors for appetitive stimuli. The final chapter of this dissertation sought to understand how old age can affect a particular facet of adaptive decision making, that is, decisions made about rewards under probabilistic contingencies. The data first establish that probabilistic decision making is in fact affected by age in that rats show higher discounting under probabilistic conditions. We found that hypoactivity in VTA neurons of aged rats may contribute to this bias. The data presented in the studies of the current dissertation provide some additional insight into how information relating to decision-relevant appetitive stimuli is processed over a broad neural circuit. Understanding how specific regions of the brain contribute to computations of decision-relevant stimuli and behavior can lend insight into how decisions are made and remembered.

### Hippocampus distinguishes contexts based on econometric variables

It has long been known that the hippocampus is essential for episodic memory and distinguishing between distinct spatial contexts (O'Keefe and Nadel, 1978; Tulving and Markowitsch, 1998). However, the hippocampus does not simply represent the environmental features of a context,

but internal drives or context-specific requirements can influence place fields representation of a context (Markus et al., 1994; Smith and Mizumori, 2006b). We investigated whether hippocampus would distinguish between contexts that were solely differentiated by changing economic variables. To do this, we recorded hippocampal place fields and theta oscillations from rats while they performed a maze-based version of the probability discounting task (Chapter 1; Tryon et al., 2017). We found that while a proportion of primary place field activity remained stable over the course of a session, a subset of place fields remapped in response to changes in the econometric variables: agency, probability and outcome. The fact that a proportion of the population did not remap is consistent with previous reports that indicate these cells are representing the features of the environment that did not change, such as the sensory features of the room and maze apparatus (Mizumori et al., 1999). In addition, our data showed that theta power was significantly elevated at the reward location, and the reward outcome of a given trial exerted the greatest predictive influence over theta oscillation amplitude. Strikingly, the theta power increases observed at the reward location were highest during rewarded trials where the risky option was chosen during the lowest probability blocks.

We also recorded VTA neurons from a separate group of rats while they performed the same maze-based probability discounting task. We found that VTA DA neurons were phasically excited by cues that informed the rat that the outcome of their choice resulted in a reward, i.e. the clicks from the feeder. Interestingly, the degree of excitation in DA neurons was the same for the first click of a large or small reward. Instead of exhibiting significantly elevated firing to the first cue that indicated imminent receipt of a large reward, the neurons instead fired to each of the four clicks separately. However, as expected, this response in DA neurons scaled with the probability of receiving the risky reward, with highest rates in response to the cue during the

lowest probability blocks, consistent with previous reports of DA neuron responses to probabilistic rewards (Fiorillo et al., 2003). By contrast, hippocampal place fields did not accumulate around the cue location, which was separated in space and time from the reward location. Instead, hippocampal place fields showed a bias to represent the reward location over all other locations of the maze. This is in line with previous research that shows that hippocampal place fields preferentially represent goal locations (Hollup et al., 2001).

These data support that the possibility that hippocampus is an important regulator of motivated behaviors. While we did not test the functional connection between VTA and hippocampus during these experiments, the VTA data support that upstream encoding of values by DA neurons can perhaps influence hippocampal processing. Importantly, the data provide evidence that hippocampus is important for distinguishing contexts that are solely defined by changes in goals and reward contingencies.

**PAG encodes appetitive stimuli**

We were initially interested in the periaqueductal gray due to its anatomical connections to the VTA. However, to warrant investigation into the functional connection between PAG and VTA, we first wanted to assess if PAG neurons encode rewarding stimuli in a similar manner to the VTA. To examine whether PAG neurons may encode rewarding stimuli, PAG neural activity was recorded while rats ran on a spatial working memory task for differential rewards (Chapter 2). Recording single neurons' firing properties with electrophysiological recording techniques and correlating those firing patterns with environmental and behavioral events has been important in providing insight into what functions particular neurons are influenced by. It was found that a subgroup of PAG neurons showed phasic response properties to reward encounters

that scaled with the size of reward received. Of reward-correlated neurons, some were reward excited and some were reward inhibited. The proportion of reward correlated neurons varied between the columns of the PAG. Reversible chemical inactivation of the PAG on the same working memory task as well as food intake tests revealed that PAG may important for consumption of food rewards, as reward consumption was found to be significantly decreased on during both experiments. These results provide some of the first evidence that the PAG may be important for processing appetitive stimuli.

An intriguing finding of one of the current studies is that a subset of PAG neurons that encode rewards conjunctively encode movement in a manner similar to VTA neurons. These neural codes could solely represent movement of the animal, but the conjunctive encoding of movement and reward in the same neurons suggest that they might reflect proximity to reward. Experience-dependent neural codes in especially the associative areas of the cortex (e.g. frontal, parietal and temporal cortices) reflect an animal's expectations for action outcomes (e.g. Duhamel et al., 1992). Since both decision and memory systems include cortical processing (and presumably expectancy for action outcomes), our lab has previously tested rats as they freely navigated on goal-directed spatial tasks that allows one to assess not only the relationship between neural responses and behavioral choices, but also between neural responses and behaviors that lead to and follow a choice. All of the decision and memory brain structures recorded show strong neural firing that correlated with rats' velocity or acceleration of forward movement including prefrontal cortex, orbital frontal cortex, parietal cortex, retrosplenial cortex, entorhinal cortex, hippocampus, amygdala, striatum, and many midbrain regions such as the VTA, substantia nigra, pedunculopontine tegmentum (PPTg), and the lateral dorsal tegmentum (LDTg) (Eschenko & Mizumori, 2007; Jo et al. 2013; Martig and Mizumori, 2011; McNaughton

et al., 1994; Norton et al., 2011; Pratt and Mizumori, 1998, 2001; Puryear et al. 2010; Redila et al., 2015; Smith et al., 2011). The essentially universal neural representation of movement speed suggests that these representations may serve as a common denominator for a general function such as planning specific actions according to their expected consequences. Since the nature of the movement representation (i.e. speed) is quite similar in all memory and decision brain areas it is not immediately obvious how these representations can reveal interactions between the two systems. At this time, the movement correlates found in the PAG warrant further investigation, but they indicate that PAG might also be important for action selection.

While our initial interest in the possibility of PAG neurons processing rewarding stimuli was because of its anatomical connections to the VTA, we did not explicitly test a functional link between the VTA and PAG. Therefore, we do not know if the phasic excitation to rewards observed in PAG neurons is a significant driver of VTA DA neuron excitation. We know other subcortical areas function to drive the distinctive firing rate properties of DA neurons in response to rewarding stimuli. For example, the laterodorsal tegmentum (LDTg) projects gulatmatergic and cholingeric afferents to the VTA and is an important driver of reinforcement (Steidl et al., 2016; Lammel et al., 2012). The PPTg is a nucleus made up of glutamategic and cholinergic neurons and is well known for its role in driving the phasic excitation of VTA DA neurons (Semba and Fibiger, 1992) and it relays sensory information about the environment and about cues that are associated with reward to the VTA (Pan and Hyland, 2005; Steckler et al., 1994). The lateral habenula (LHb) is a structure that exerts a major inhibitory influence on the DA neurons of the VTA via the mostly GABAergic neurons of the rostromedial tegmental nucleus (RMTg), (Jhou et al., 2009; Bromberg-Martin and Hikosaka, 2011). The firing properties of PAG neurons in response to appetitive stimuli recorded in the current dissertation are more in

line with the properties of structures like the PPTg and the LDTg. However, it may be that the reward excited neurons in the PAG are GABAergic interneurons inhibiting PAG defensive behavioral output rather than driving excitation of DA neurons in the VTA. Therefore, future work needs to identify the neuronal subtype of reward-excited neurons in the PAG and assess if that subgroup projects to the VTA DA neurons.

**Risk-based decision making is affected over the lifespan and is not related to hippocampal function**

As organisms age, loss of physiological integrity in various tissues can affect multiple facets of the health of an organism (Lopez-Otin et al., 2013). However, in the absence of disease, the brain maintains a surprising level of its integrity in structure and function (Whitbourne, 2002). Instead, synaptic alterations in vulnerable circuits define brain aging rather that substantial neuron loss (Hof and Morrison, 2004). Importantly, subtle changes in these circuits is known to affect decision and memory functions, most commonly examined by probing synaptic changes in hippocampal and prefrontal circuits (Morrison & Baxter, 2012; Barense et al., 2002; Dumitriu et al., 2010). However, whether changes in midbrain regions such as the ventral tegmental area could contribute to changes in cost-benefit decision making is not known. Therefore, we wanted to examine how probabilistic decision making was affected with age, how VTA neural processing of rewarding stimuli differed between young and aged rats' neurons. Finally, we wanted to examine whether any observed changes were related deficits in hippocampal memory (Chapter 3), as we know these circuits are functionally linked (see Introduction). We tested aged and young rats choice behavior between large and small rewards on a probability discounting task that tested rats' preference for a lever that led to a large reward with varying probability (the risky option) or a lever that lead to a small reward 100% of the time (the certain option). The

probabilities associated with the risky lever were 100%, 50%, 25%, and 12.5%. The central question was how aged rats choices for risky rewards would differ from their younger counterparts as previous research indicates aged rats would show a preference for the small, certain reward over the risky one, even when the outcome would be better if the risky option was selected (Mather et al., 2012; Lee et al., 2008). The same animals were also tested on a water maze, a hippocampal dependent task, to quantify the extent of their spatial memory deficits and see if hippocampal deficits were linked to alterations on probability discounting.

The current data show that aged rats display decreased choice of the large risky reward, i.e. more discounting on the probability discounting task, reflecting an age-related change in risk-based decision making, in line with increased risk-aversion observed in older humans (Lee et al., 2008). We found that aged rats show decreased win-stay behavior compared to their younger counterparts which contributed to the increased discounting observed. It is thought that this reflects a deficit in maintaining a choice after positive reinforcement, which is supported by previous data that aged rats have a deficit in repeating a choice that was previously rewarded (Means and Holsten, 1992).

To examine whether changes in dopamine neurons of the VTA might contribute to the observed changes in risk based decision making, DA neurons of the VTA were recorded in young and aged rats while they performed the probability discounting task. Previous research supports that alterations in DA function can contribute to changes in cost-benefit decision-making strategies. For example, dopamine alteration can affect delay- and effort-based cost-benefit decision making (Floresco, Tse, and Ghods-Sharifi, 2007). Additionally, we know that dopamine neurons and subsequent dopamine release are intrinsically linked to the value of probabilistic rewards during risky decision making tasks (Fiorillo et al., 2003; Sugham et al.

2012). The preliminary data show a striking reduced responsiveness of aged VTA neurons to reward-predictive and rewarding stimuli. Additionally, we found a significantly reduced rate and responsiveness of putative DA neurons in aged VTA. However, the neurons classified as dopaminergic in the aged rats may not be truly dopaminergic due to the possibility that the waveform characteristics have changed with age (Rollo, 2009; Branch et al., 2014). Future work should aim characterize the identity of recorded VTA neurons in aged rats to elucidate if the blunted responses are due to changes in dopamine neuron function specifically or VTA neurons in general.

Finally, we examine the relationship between hippocampal function and probability discounting behavior in young and aged rats. Interestingly, we did not find a significant relationship between water maze performance and risky choices. This is consistent with previous similar studies that found no relationship between the two types of behavioral performances, even in advanced age (Gilbert et al., 2012; Samson et al., 2015). Therefore, there is insufficient data at this time to link spatial working memory decline in age with increased probability discounting.

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**Curriculum Vitae**

## Education

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|---|---|
| September 2011-June 2017 | PhD in Psychology, Behavioral Neuroscience -University of Washington |
| September 2006-May 2010 | Bachelor of Arts in Psychology, Minor in Biology -California State University, Sacramento - *Cum Laude* |

## Research Experience

| | |
|---|---|
| July 2011-Present | Graduate Research Assistant – University of Washington, Department of Psychology, Behavioral Neuroscience |
| June 2010-June 2011 | Laboratory Intern -Institute for Pediatric Regenerative Medicine, Shriners Hospital for Children, Northern California |
| August 2009-June 2011 | Laboratory Assistant -California State University, Sacramento |

## Awards

| | |
|---|---|
| Spring 2017 | Hunt Endowed Fellowship for Graduate Students in Psychology |
| May 2013-March 2017 | Predoctoral Fellowship-Genetic Approaches to Aging Training T32 grant |
| 2013 | National Science Foundation Graduate Research Fellowship-Honorable Mention |
| 2013 | University of Washington, Department of Psychology Graduate Student Service Award |

## Publications

**Tryon, VL,** & SJY Mizumori. (2017). A novel role for the periaqueductal gray in consummatory behaviors. *Submitted to J. Neuroscience.*

**Tryon, VL,** Penner, MR, Heide, SW, Larkin, JL, & SJY Mizumori. (2017). Hippocampal neural activity reflects the economy of choices during goal-directed navigation. *Hippocampus.*

**Tryon, VL,** Mizumori, SJY, & MM Morgan. (2016). Analysis of morphine-induced changes in the activity of periaqueductal gray neurons. *Neuroscience, 335,* 1-8.

Mizumori, SJY, & **Tryon, VL.** (2015). Integrative hippocampal and decision-making neurocircuitry during goal-relevant predictions and encoding. Progress in Brain Research, 219: 217-242. PMID: 2607224

**Tryon, VL**, Kim EU, Zafar TJ, Unruh AM, Staley SR, & JL Calton. (2012). Magnetic field polarity fails to influence the directional signal carried by the head direction cell network and the behavior of rats in a task requiring magnetic field orientation. *Behavioral neuroscience, 126(6),* 835-844.

## Conference Presentations

**Tryon, VL** & SJY Mizumori. (2016) "A novel role for the periaqueductal gray in consummatory behaviors."
Society for Neuroscience Annual Conference- San Diego

**Tryon, VL**, King, HO, Long, JM, Rapp, PR & SJY Mizumori. (2016) "Increased risk aversion with age on a probability discounting task."
AGE meeting- Seattle

**Tryon, VL**, King, HO, Long, JM, Rapp, PR & SJY Mizumori. (2015) "Increased risk aversion with age on a probability discounting task."
Society for Neuroscience Annual Conference- Chicago

SJY Mizumori, **Tryon, VL,** Penner, MR, & Larkin, JL. (2014). "Perceived risk and agency impact hippocampal place field organization during performance of a probability discounting maze-based task."
Society for Neuroscience Annual Conference-Washington, D.C

**Tryon, VL**, Foreman, E, & SJY Mizumori. (2013) "Reward-sensitive neural responses in the periaqueductal gray during a spatial working memory task."
Society for Neuroscience Annual Conference- San Diego

**Tryon, VL**, Brumm, LE, Penner,MR, & SJY Mizumori. (2012) "A role for the periaqueductal gray in reward-related processing."
Society for Neuroscience Annual Conference- New Orleans

Penner, MR, Larkin, JL, **Tryon, VL**, Jaramillo, D, & SJY Mizumori. (2011). "Possible role for periaqueductal gray in reward-related processing."
Society for Neuroscience Annual Conference-Washington, D.C

**Tryon, VL**, Kim EU, Zafar TJ, Unruh AM, Staley SR, & JL Calton. (2010). "Head direction cell activity recorded during alteration of geomagnetic field polarity."
Society for Neuroscience Annual Conference- San Diego

Miller, EN, **Tryon, VL**, & V Martinez-Cerdeno. (2010).“Molecular characteristics and proliferative potential of precursor cells and their regulation by neurotransmitters in the embryonic spinal cord.”
U.C. Davis, Institute for Pediatric Regenerative Medicine Annual Research Symposium

## Teaching Experience

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|---|---|
| Fall 2008; Spring 2009 | Teaching Assistant, Psychology - California State University, Sacramento |
| Winter 2013; Spring 2013; Fall 2013; Winter 2014; Fall 2014; Winter 2015; Winter 2016 | Teaching Assistant, Psychology- University of Washington |

## Professional Memberships

Society for Neuroscience- 2010 -Present

American Psychological Association-2012-2013

## Service

Graduate Student member of Behavioral Neuroscience Faculty Search Committee 2012-2013; 2013-2014