A Role for Hippocampal and Midbrain Neural Processing in Context-Dependent Spatial Memory

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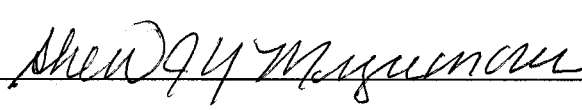

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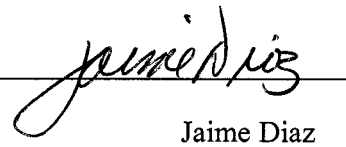

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Abstract

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The ability to discriminate contextual situations is central for organisms to predict events and the outcome of behavior. This function is thought to be the byproduct of an allocentric spatial reference framework generated by the hippocampus. It is commonly assumed that hippocampal place cells (i.e., neurons that are activated when the animal occupies a particular location in the environment; the place field), underlie this function. However, it has been difficult to ascertain which aspects of hippocampal place fields are critical to perform spatial memory tasks. By recording hippocampal place cell activity while rats performed a hippocampal-dependent spatial memory task, I demonstrated that the overall specificity of place fields appears to be critical for spatial memory functions. Specific hippocampal representations of the context may be sculpted by the neuromodulator dopamine released from the ventral tegmental area (VTA). Although the activity of VTA dopamine neurons is thought to signal an error in the prediction of salient events (particularly rewards) within a given context, it has not been demonstrated that VTA neural activity is modulated by contextual information. Therefore, I recorded the activity of VTA neurons as rats performed a similar spatial memory task under varying contextual conditions. I found that the activity of reward-related VTA neurons was, indeed, gated by contextual information and exhibited reward prediction error-related activity when rewards were unexpectedly altered. However, it appeared that not

all of these were dopamine neurons. This is consistent with recent reports that dopamine neurons may not generate prediction errors, per se, but may be generated by 'upstream' brain areas. Therefore, I recorded the activity of neurons in the mesencephalic reticular formation (MRNm), which sends excitatory (i.e., glutamatergic) inputs to VTA. I discovered that the majority of MRNm neurons exhibited reward-related activity and that they displayed prediction error-related activity similar to VTA neurons. Together, this dissertation provides new evidence that MRNm may, at least participate with VTA in signaling other brain areas, such as hippocampus, that expected consequences of behavior have changed. This likely enables a new representation of the context to be formed and stored into long-term memory.

Table of Contents

List of Figures

List Of Tables

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Dedication

To my parents, Gary and Marcia Puryear

You taught me to believe in myself.

For Wendy & Aslen

Chapter 1: Background and Introduction

The role of hippocampus in learning and memory

The hippocampus, a brain structure found within the medial temporal lobe, has long been known to be important for forming new long-term episodic memories. That is, hippocampus is necessary to convert a short-term representation of daily events into a form that endures long passages of time. Episodic memory is considered to be a particularly rich and dynamic form of memory which entails the people and objects, places, and chronology that comprised the event (i.e., the 'what', 'where, and 'when'), as well as a form of 'mental time travel' that essentially allows the individual to relive the memory during recall (Tulving, 2002). Perhaps the most well known figure at the epicenter of the past 50 years of research on the mnemonic functions of hippocampus is patient H.M. In 1953, H.M. underwent surgery to remove portions of the medial temporal lobe, including hippocampus, on both sides of his brain to alleviate the chronic and intractable epilepsy he suffered from. Although the severity of his epilepsy was dramatically reduced, H.M. was left with profound anterograde episodic amnesia. That is, although he was able to remember some new events he experienced for a brief time (i.e., short term memory), he was unable to commit those new events into a long lasting form (i.e., long term memory) (Scoville and Milner, 1957). The memory deficit seen in H.M. appeared to be limited to episodic memories, since he was able to learn new motor skills and some simple conditioning tasks, and displayed normal perceptual priming abilities. Therefore, it appeared that the hippocampus was important for forming memories that incorporates events that occurred at particular times and places. Further research has demonstrated that the human hippocampus is not only important for

recalling past events, but may also be important for imagining events and situations that could happen in the future (Tulving, 1985; Tulving and Markowitsch, 1998; Addis et al., 2007; Hassabis et al., 2007). Although much of the human clinical work has solidified a prominent role for hippocampus in episodic memory functions, it has been convincingly demonstrated that hippocampus also plays an important role in spatial learning.

As a prominent component of episodic memory, spatial memory entails a complex association between the relative positions of stimuli, particularly visual, in the environment. Hippocampal lesions produce profound spatial learning impairments in humans, non-human primates, and rodents (Morris et al., 1982; Walker and Olton, 1984; Gaffan and Harrison, 1989; Astur et al., 2002; Lee and Kesner, 2003). Much of our understanding of the role hippocampus may play in spatial memory has come from single unit recording studies in freely behaving animals. In the rodent, most hippocampal pyramidal cells (i.e., place cells) dramatically increase their firing rates when the subject occupies specific locations (i.e., place fields) in the environment (O'Keefe and Dostrovsky, 1971; Muller et al., 1987), indicating that spatial information is represented at the single-cell level in rodent hippocampus. Similarly, primate hippocampal neurons have been shown to fire in relation to the subject's spatial view (Georges-Francois et al., 1999). In humans, functional imaging studies have demonstrated increased hippocampal activity when subjects perform spatial navigation tasks (Maguire, 1997; Maguire et al., 1997), and there is some evidence that single cells in the human hippocampus also exhibit location-specific activity, akin to that observed by rodent hippocampal place cells (Ekstrom et al., 2003). Numerous studies in rodents have documented that the locations of many place fields are bound to visuo-spatial cues in the environment. For example, if

the visuo-spatial cues in an environment are rotated by 90°, most place fields will rotate by the same amount, maintaining their same positions relative to the new orientation of visuo-spatial information (Muller and Kubie, 1987). Furthermore, blockade of NMDA receptor-dependent long-term potentiation (LTP), a form of synaptic plasticity thought to underlie various forms of learning (Martin et al., 2000), has been shown to impair spatial learning and disrupt the formation and stability of place fields in a novel environment (Morris et al., 1986; McHugh et al., 1996; Tsien et al., 1996; Kentros et al., 1998). These data support early propositions that the place cell was the fundamental unit of a spatial representation of the environment that formed a "Cognitve Map" that could be used for accurate navigation (O'Keefe and Nadel, 1978).

In addition to the obvious spatial nature of hippocampal neural activity, several lines of research have indicated that hippocampal neural activity is modulated by many types of non-spatial information. Hippocampal neurons have been shown to fire relative to events that occur in particular places (Wood et al., 1999), changes in reinforcement conditions (Holscher et al., 2003; Smith and Mizumori, 2006a), and the particular behavioral strategy used to obtain reward (Yeshenko et al., 2004; Eschenko and Mizumori, 2007). It has also been documented that past and future behaviors can have strong influences on place cell firing patterns (Wood et al., 2000; Ferbinteanu and Shapiro, 2003; Foster and Wilson, 2006; Diba and Buzsaki, 2007). Thus, hippocampus appears to encode a multifaceted construct about what, when, and where events occur. This has led some to propose that the fundamental role of hippocampus may be to form a complex informational construct referred to as the 'spatial context' (Mizumori et al., 1999b; Mizumori et al., 2000; Jeffery et al., 2004).

Before continuing, it is important to clarify what is meant by the term context. Historically, it has been defined as the set of sensory stimuli (i.e., odors, cues, sounds, etc) particular to a situation. This is in part because these stimuli tend to be more stable and therefore better predictors of events that have, or will occur in that environment. A role for hippocampus in processing context information during learning emerged in the 1970's from Pavlovian and instrumental conditioning studies (Hirsh, 1974; Winocur and Olds, 1978). Since then, there has been substantial documentation that hippocampus is critical for learning contextual information (for reviews see Holland and Bouton, 1999; Anagnostaras et al., 2001; Smith and Mizumori, 2006b). Specifically, it has been shown that animals with impaired hippocampal function are unable to acquire context-dependent fear responses (Kim and Fanselow, 1992; Phillips and LeDoux, 1994). Furthermore, lesions to hippocampus or entorhinal cortex, the main source of cortical input to hippocampus (Witter et al., 2000), block the normal decrement in responding when animals are required to perform a previously learned task in a new context, indicating that they are insensitive to changes in contextual information (Penick and Solomon, 1991; Freeman et al., 1997). Thus, the findings that spatial, behavioral, temporal, and mnemonic information can control the activity of hippocampal place cells and the role hippocampus appears to play in forming context-specific memories has led to theories that hippocampus may engage in computations important for determining whether one context is different from another (Mizumori et al., 1999a; Vinogradova, 2001). This context discrimination would then facilitate the recall of the meaning of specific contexts such that the animal could engage in appropriate behaviors upon subsequent exposures to them. Similarly, if aspects of a familiar context are significantly altered (i.e., reward

contingencies unexpectedly change), a new representation could be formed and stored into long-term memory, again in an effort to facilitate the selection of context-appropriate behaviors upon future encounters with the new context.

A role for ventral tegmental area in context processing

Along with substantia nigra *pars compacta* (SNc), the ventral tegmental area (VTA) of the midbrain is one of the main sources of dopamine in the brain. Dopamine is considered a powerful neuromodulator important for several learning and memory functions, the most widely studied of which, is its role in reinforcement learning. In 1954, it was observed that rats will readily learn to press a lever for direct, intracranial self-stimulation (ICSS) of the medial forebrain bundle (MFB) (Olds and Milner, 1954). It is now thought that this learning is likely due to the activation of dopaminergic fibers, since this effect can be mimicked by direct infusion of dopamine into in a target area of the MFB, the nucleus accumbens (NAc), and blockade of dopamine receptors in NAc prevents animals from learning the operant response (Wise and Rompre, 1989). As such, much of the research on the functions of dopamine has been directed towards its role in reward processing and reinforcement learning (Berridge and Robinson, 1998; Schultz, 2002; Wise, 2004; Fields et al., 2007).

Much of what is understood about the role of dopamine in reinforcement learning is the product of single cell recording studies in animals performing conditioning tasks. In these situations, it is commonly observed that dopamine neurons are activated by the presentation of unconditioned stimuli, such as a reward, if its delivery is not expected. Furthermore, the magnitude of the response appears to be proportional to the magnitude

of reward received, as larger amounts of reward elicit more robust firing (Tobler et al., 2005). However, as the subject begins to learn about cues that reliably predict the delivery of the reward (i.e., tones, cue lights), dopamine neurons gradually loose their response to the delivery of the reward, and at the same time, gradually develop a response to the presentation of the cue. In addition, if an expected reward is not delivered following presentation of a predictive cue, the firing rate of dopamine neurons is suppressed (Hollerman and Schultz, 1998; Bayer and Glimcher, 2005; Pan et al., 2005; Tobler et al., 2005; Roesch et al., 2007). These data have led to the hypothesis that the activity of dopamine neurons represents an error in the prediction of reinforcement, such that activation of dopamine neuronal activity by an unpredicted reward represents a positive reward prediction error, and inhibition of dopamine neuronal activity by the absence of an expected reward represents a negative reward prediction error. These signals are thought to function as a teaching signal for other areas of the brain, particularly NAc, that are able to engage in corrective behaviors in an attempt to learn the new task contingencies (Schultz and Dickinson, 2000; Wise, 2004; Fields et al., 2007).

Related to its prominent role in reinforcement learning, dopamine is also important for hippocampal-dependent forms of learning. Hippocampus receives most of its dopaminergic afferents from VTA, which innervates the entire dorso-ventral extent of hippocampus, whereas innervation from SNc dopamine neurons appears to be limited to dorsal hippocampus (Gasbarri et al., 1997). In humans, greater correlated activation of VTA/SNc and hippocampus predicted better recall of a hippocampal-dependent task, suggesting that greater activation of midbrain dopaminergic areas enhances memory formation (Wittmann et al., 2005). Accordingly, intra-hippocampal administration of D1

or D2 dopamine receptor agonists can improve short-term spatial memory (Packard and White, 1991). Furthermore, dopamine agonists can ameliorate the decline in spatial learning commonly observed in aged animals (Hersi et al., 1995; Bach et al., 1999). Conversely, elimination of hippocampal dopamine innervation by direct infusions of 6 hydroxydopamine (6-OHDA), which causes selective cell death of innervating dopamine neurons (Sauer and Oertel, 1994; Przedborski et al., 1995), impairs spatial learning in the Morris Water Maze (Gasbarri et al., 1996). Furthermore, mice lacking D2 and D3 dopamine receptors display impaired spatial working memory abilities (Glickstein et al., 2002). A role for dopamine in hippocampal-dependent mnemonic functions is not limited to spatial learning, as it appears that dopamine is also important for contextual processing. Several studies have documented increased dopamine release in several limbic areas, such as NAc (Legault and Wise, 2001), prefrontal cortex (Feenstra et al., 1995; Feenstra and Botterblom, 1996), and hippocampus (Ihalainen et al., 1999) when animals are placed into a novel context.

This novelty-induced dopamine release plays an important role in enabling synaptic plasticity mechanisms in hippocampus that likely underlie the formation of representations of the novel context. It has been demonstrated that exposure to a novel environment reduces the threshold for inducing LTP in the CA1 region of hippocampus (Li et al., 2003). Furthermore, the metaplasticity-like effects (Bienenstock et al., 1982) observed in these situations are blocked if the animal is given a D1 antagonist prior to exposure to the new context (Li et al., 2003; Lemon and Manahan-Vaughan, 2006). Accordingly, the maintenance of hippocampal LTP is blocked *in vitro* by pretreatment with dopamine antagonists (Frey et al., 1990; Frey et al., 1991). In the freely behaving

animal, dopamine receptor manipulation has been shown to alter context-dependent hippocampal place cell activity. For example, systemic administration of a D1/D5 dopamine receptor agonist and antagonist can increase and decrease, respectively, the stability of place fields in mice foraging for food in a familiar environment (Kentros et al., 2004). Furthermore, D1/D5 antagonism was shown to alter place field reliability and specificity, but only under changing contextual conditions (Gill and Mizumori, 2006). Thus, it appears that dopamine is important for stabilizing hippocampal place fields under conditions of contextual instability.

The ventral tegmental area is more than the sum of its dopamine

Although the overwhelming majority of attention VTA has received has been due to its dopamine content, it is somewhat surprising that only ~55% of VTA neurons express tyrosine hydroxylase (TH), a common marker of dopamine neurons, where as SNc is comprised of ~88% dopamine neurons (Margolis et al., 2006). In addition to neurons that synthesize and release γ-amino butyric acid (GABA, a major inhibitory neurotransmitter), VTA has recently been found to contain a population of glutamate neurons (Yamaguchi et al., 2007; Nair-Roberts et al., 2008a). This supports the notion put forth that the actions VTA neurons have in their target areas are, at least, partially due to the release of glutamate (Sulzer and Rayport, 2000; Chuhma et al., 2004; Lavin et al., 2005).

These data are particularly important for two main reasons. First, although dopamine has profound effects on hippocampal function and synaptic plasticity (discussed above), only 10-18% of VTA afferents to hippocampus are dopaminergic

(Gasbarri et al., 1994). Therefore, it is likely that the remaining 80-90% of VTA afferents to hippocampus, be they glutamatergic and/or GABAergic, play important roles in hippocampal-dependent learning and memory functions. Second, VTA unit recording studies performed in freely moving animals have attempted to identify a given neuron as dopaminergic on the basis of its electrophysiological properties (i.e., firing rate and duration of the action potential) and changes in firing rate following manipulation of D2 receptors, which are presynaptic autoreceptors on dopamine neurons (Grace and Bunney, 1983). Thus, putative dopamine neurons are considered to exhibit long action potentials (between 1.5 and 2.5 msec, depending on amplifier filter settings and electrode impedence) and decrease or increase firing rates following application of D2 agonists or antagonists, respectively (Grace and Bunney, 1983). Although, this is a source of current debate, a recent study has called into question whether this commonly used method is valid for recordings of VTA neurons (Margolis et al., 2006). According to Margolis et al. (2006), ~45% of neurons in the rodent VTA that pass the aforementioned dopamine neuron criteria do not test positive for the presence of TH, indicating that they may not be dopamine neurons. This is particularly important for interpreting reward prediction error-related activity of VTA neurons. Given the current debate as to the validity of identifying dopamine neurons in freely behaving animals, one has to consider the possibility, at least in rodents, that reward prediction error-related activity may also be exhibited by non-dopamine neurons.

It is without question that many other brain areas participate in appetitive learning functions. However, it is commonly thought that dopamine neurons are the source of positive and negative reward prediction errors (Schultz and Dickinson, 2000). This is in

part because there is little evidence that neurons in other reward-related brain areas display the same activity relative to unexpected presentation and omission of rewards (Schultz and Dickinson, 2000). However, recent studies have begun to provide evidence that reward prediction error-related activity may not be generated by dopamine neurons, *per se*, but may be generated in brain areas 'upstream' of VTA.

For example, the activity of neurons in the pedunculopontine tegmental nucleus (PPTg), which is a potent regulator of VTA and SNc burst firing (Floresco et al., 2003), has recently been shown to vary depending on whether the animal received expected rewards (Kobayashi and Okada, 2007), indicating that PPTg could be involved in generating the prediction error signals observed in dopamine neurons. In addition, neurons in the lateral habenula (lHb), a source of inhibitory GABAergic input to VTA and SNc (Herkenham and Nauta, 1979; Christoph et al., 1986) exhibit short-latency inhibitory and excitatory responses upon unexpected presentation and omission of rewards, respectively (Matsumoto and Hikosaka, 2007). Therefore, it is possible that, upon presentation of an unexpected reward, the inhibition on dopamine neurons provided by lHb neurons is lost, thereby allowing them to fire. The opposite would be the case when an expected reward is omitted. This signal provided by lHb would not, however, be sufficient to drive the excitatory responses seen in dopamine neurons upon delivery of an unexpected reward (i.e., the positive reward prediction error). As such, it has recently been shown that VTA receives the majority of its excitatory, glutamatergic innervation from subcortical sources, including structures such as the mesencephalic reticular formation (MRNm) (Geisler et al., 2007). Thus, any of these subcortical structures, in

combination with inhibitory inputs from lHb, could be in a strategic position to drive the excitatory component of the prediction error signal in dopamine neurons.

The overall goal of the work presented in this dissertation is to provide some insight into the neural mechanisms of context-dependent memory functions. First, I will describe work highlighting aspects of hippocampal place cell activity that are important for context-dependent spatial memory. Second, I will describe evidence at the single cell level that VTA is indeed involved in processing changes in context information. Finally, I provide some novel evidence that neurons in MRNm may be a component of a larger system involved in generating the patterns of reward-related activity observed in VTA neurons.

Chapter 2: Hippocampal Place Cells and Context-Dependent Spatial Memory

Hippocampal damage in several animal species impairs the ability to learn tasks that depend on the use of allocentric spatial information (Morris et al., 1982; Gaffan and Harrison, 1989; Astur et al., 2002). Furthermore, numerous electrophysiological studies have demonstrated that the firing rates of hippocampal pyramidal cells (place cells) are strongly modulated by the spatial location (place field) of the rat within the recording environment (O'Keefe and Dostrovsky, 1971). The entire area of the testing environment is represented by subpopulations of hippocampal place cells, and the moment-to-moment spatial location of the subject can be reliably predicted by the activity of neural population codes (Wilson and McNaughton, 1993). Furthermore, the spatial firing patterns of place cells (e.g., locations of their place fields) are sensitive to changes in the spatial environment such that manipulations of spatial cues can cause alterations in the locations of place fields (i.e., they reorganize) (Muller and Kubie, 1987; Quirk et al., 1990; Markus et al., 1994). Despite this wealth of evidence, it has not been established how the activity of individual hippocampal place cells plays a role in spatial memory.

Some studies have investigated the relationship between place cell firing and task performance, but these studies have produced mixed conclusions. The majority of these studies have examined the relationship between changes in the locations of place fields and changes in performance of spatial tasks. For instance, it has been shown that manipulations of the visual environment that caused place fields to be out of register relative to their standard configuration also resulted in a decrease in rats' performance on a continuous alternation task (Lenck-Santini et al., 2001). In contrast, other manipulations that cause a robust reorganization of place fields do not always affect rats'

performance of hippocampal-dependent spatial tasks (Cooper and Mizumori, 2001; Jeffery et al., 2003). These data suggest that, at best, the relationship between the locations of place fields (and changes therein) and the performance of spatial tasks is not consistent. This notion is at odds with the overwhelming evidence that hippocampal cells are important for accurate performance of spatial learning tasks (O'Keefe and Nadel, 1978).

This study addressed the extent to which aspects of place fields other than their locations (i.e., specificity or reliability of place fields) may relate more directly to the subject's performance of hippocampal-dependent spatial tasks, since they may be more indicative of the overall visuo-spatial acuity of the hippocampal representation. Hippocampal place cells were recorded while rats performed a spatial working memory task, and then changes in task performance were compared with changes in place field characteristics in response to a visuo-spatial change in the testing environment.

METHODS

Subjects

Hippocampal single units were recorded from 13 adult (4-6 months old) male Long-Evans rats. Rats were housed individually and allowed 3-5 days to acclimate to the colony room prior to being reduced to 85% of *ad lib* feeding weights. All rats had unlimited access to water throughout the experiment. All animal care and use was conducted according to University of Washington's Institutional Animal Care and Use Committee guidelines.

Rats were habituated to the testing environment and then trained to perform a win-shift spatial working memory task on an eight-arm radial maze using procedures

reported previously (Mizumori et al., 1989; Cooper and Mizumori, 2001; Pratt and Mizumori, 2001). Briefly, the end of each arm was baited prior to the start of each trial with 3 drops of chocolate milk. Each trial started with a study phase in which four of the eight arms were individually and sequentially presented to the rat in a predetermined random order. Immediately after presentation of the fourth arm, the test phase began by making all arms accessible. The trial ended once all eight arms were visited; entries into previously visited arms were classified as errors. In order to promote the use of a spatial navigation strategy, several distinct and prominent cues were attached to the black curtains that surrounded the maze. Once rats performed 15 trials (inter-trial interval = 2 min) in approximately one hour for seven consecutive days, recording electrodes were surgically implanted into dorsal hippocampus. After rats recovered from surgery (approximately 1 week), they were re-trained on the task.

Surgical procedures and histology

Details concerning the construction of recording stereotrodes and microdrives and surgical procedures can be found in previous reports (McNaughton et al., 1983b; Mizumori et al., 1989). Briefly, stereotrodes were constructed by twisting together two laquer-coated tungsten wires (California Fine Wire) and passing them through a 30 ga stainless steel guide cannula. Three stereotrodes were then secured to each microdrive (one per hemisphere) with epoxy. Rats were anesthetized with sodium pentobarbital (Nembutal; 40 mg/kg I.P., followed by 0.05 ml supplemental doses as needed) and given atropine sulfate (5.0 mg/kg I.P.) to alleviate respiratory distress. The stereotrode microdrives and reference and ground electrodes were implanted according to previous procedures (Mizumori et al., 1989; Cooper and Mizumori, 2001). The stereotrodes were

stereotaxically implanted above dorsal hippocampus according to the following coordinates (Swanson, 1998): +2.5 to +4.5 mm posterior to bregma, ±2.0-2.5 mm lateral, and 1.7 mm ventral to the brain surface. Reference electrodes were and the ground screw was implanted into the skull. Rats were then given one week of free feeding to fully recover from surgery before being placed back on food-restriction to begin experimental procedures.

Once the electrodes were lowered through the entire dorsal-ventral extent of dorsal hippocampus at the conclusion of the experiment, rats were given an overdose of sodium pentobarbital and transcardially perfused with a 0.9% buffered NaCl solution, followed by 10 % formalin. The electrodes were retracted and the brain was removed and allowed to sink in a 30% formal-sucrose solution. Forty-micrometer coronal sections were sliced through dorsal hippocampus with a cryostat. The sections were then stained with Cresyl violet, and the recording locations were histologically verified by comparing electrode depth measurements at the time of recording with reconstructions of the electrode tracts.

Single-unit recording

Once rats recovered from surgery, they resumed performance of the spatial working memory task. Prior to each session, rats were connected to the recording equipment by a pre-amplification headstage containing 16 field effect transistors and a pair of infrared diode arrays used to track the animal's position and directional heading. All stereotrodes were checked daily for spontaneous neural activity. If no clear neural activity was encountered stereotrodes were lowered in approximately 25 μm increments (up to 175 μm per day) until clear, isolatable units were observed. The animal's position

and electrophysiological data were recorded on either the Datawave Discovery or Neuralynx Cheetah data acquisition systems. In both cases, the locations of animals' position were monitored by an infrared video camera mounted to the ceiling above the maze and recorded via automatic tracking systems (position data was sampled at 20 and 30 Hz, respectively). Single unit activity was recorded simultaneously and independently on each wire of the stereotrode. Incoming signals were amplified (3,000-10,000 times), filtered between 600 Hz and 6 kHz, and passed through a window discriminator that triggered a 1 msec sampling period when an impulse from either channel passed a user-defined threshold. The Datawave and Neuralynx acquisition systems sampled the neural data at a frequency of 32 kHz.

Single units were isolated from the multiunit records using cluster-cutting routines. The Datawave Discovery software package contained a cluster-cutting routine, whereas spike data acquired via the Neuralynx acquisition system were separated using a custom version of MClust (A.D. Redish). Each software program calculated multiple waveform parameters including peak to valley amplitudes and spike widths (time between the peak and valley of the action potential) for each sample from all stereotrodes. In addition, a template-matching algorithm (written by C. Higginson) was used offline to facilitate separation of unique spike waveforms. We only included cells with a signal-to-noise ration of at least 3:1 and which exhibited stable clusters throughout the recording session.

Spatial working memory task

Each recording session consisted of two blocks of five trials each. During the first block of trials, rats performed the spatial working memory task with the extra-maze

cues in their normal configuration (baseline trials). Following completion of the 5th trial, rats performed a second block of five trials with the maze room lights extinguished (dark trials), thereby eliminating all visuo-spatial context information. For comparison, control sessions in which the lights remained on throughout the two blocks of trials were also included. Rats remained on the maze and connected to the tether throughout the duration of the recording session.

Hippocampal neurons can be readily classified as either complex spike (CS) cells (pyramidal cells) or interneurons based on their unique spike characteristics. CS cells have broader spikes (>300 μsec from peak to valley) and typically exhibit lower firing rates than interneurons. In addition, CS cells fire in burst patterns of three to four action potentials. In order for a cell to be classified as a place cell, it had to first be classified as a CS cell as described above. Second, the cell had to have a specificity score greater than 3.0 and a reliability score greater than 50% (these terms are defined below) in at least one of the two blocks of trials. Also, only place cells with firing fields located on the maze arms (as opposed to the center of the maze) were included in these analyses. In contrast to CS cells, interneurons have narrower spikes (<300 μsec peak to valley) and fire at higher firing rates. Hippocampal interneurons were excluded from all analyses.

Data analysis

The performance of rats was assessed by calculating the average number of errors during each block of five trials. The average firing rate for all cells was determined for each block of trials. In order to evaluate spatial firing patterns, several different parameters were calculated for each cell. The *specificity* of spatial firing was calculated as the average firing rate on the arm associated with the highest firing rate divided by the

average firing rate on all other arms for each block of trials. The *reliability* of spatial firing was calculated as the percentage of trials in which the cell showed its highest firing rate on the arm with the highest average firing rate for the block of trials. A given place cell was not required to have a place field in both blocks of trials. In instances in which a cell lost or gained a place field, the specificity and reliability measures were still calculated based on the arm associated with the highest firing rate.

In order to quantify the effects of lighting condition on rat's performance and place field properties (place field reliability, specificity, in- and out-of-field firing rates, and size), difference scores (DS's) were calculated according to the following formula: $DS = (X_{dark}-X_{light})/(X_{dark}+X_{light})$. These DS's reflect the change in each of these measures relative to the first block of trials and can range from -1 to +1. Negative and positive values represent decreases and increases, respectively, in each parameter for the second block of trials. A *spatial correlation* score assessed the effects of lighting conditions on the spatial firing patterns of place cells by calculating a Pearson's correlation (r) for the firing rates in commonly visited pixels across the two blocks of trials. We then computed oneway ANOVA's ($\alpha = 0.05$) to determine if lighting condition had effects on DS's for each of the above parameters and spatial correlation scores. In order to examine potential confounding variables that influence place cell firing properties, such as running speed (Czurko et al., 1999), we calculated the mean amount of time rats spent on each arm in each block of trials. Changes in time per arm choice were also computed in terms of DS's.

Finally, the relationship between changes in the rat's performance of the spatial working memory task and the average changes in place field properties following

changes in lighting condition was investigated. Since this analysis was aimed at examining changes in populations of place cells, this analysis was performed on datasets containing at least two simultaneously recorded place cells. More specifically, the average difference scores for place field specificity and reliability, and spatial correlation scores were correlated with performance difference scores for individual recording sessions.

RESULTS

Histological examination of the locations of recording electrodes indicated that electrodes passed through the CA1, hilar CA3, and dentate gyrus (DG) regions of dorsal hippocampus. We recorded a total of 72 place cells (n's: CA1 = 24, CA3 = 8, DG = 39, the location of 1 place cell was not able to be identified). The relatively small number of CA3 place cells (control = 4, light-dark = 4), precluded valid statistical comparison between responses of CA1 and CA3 place cells for control and light-dark manipulations. Additionally, due to the relatively small sample size (Controls: CA1 = 12, DG = 18; Light-Dark: CA1 = 12, DG = 21) and the number of statistical tests required to perform the comparisons (increasing the occurrence of Type 1 errors), we were not able to determine whether there were differences in how CA1 and DG cells responded in control and light-dark manipulations. Therefore, all place cells were grouped together in all analyses.

Consistent with previous reports (Mizumori and Williams, 1993), there was a significant overall main effect of lighting condition on rats' performance ($F[1, 45]= 5.90$, $p < 0.02$) in that performance difference scores were significantly lower for the dark manipulation when compared to controls. That is, rats made significantly more errors

after darkness was imposed than following the control condition (Fig. 1.1a and Table 1). A more detailed analysis of rats' behavior on the maze indicated that, although rats spent significantly more time per arm choice during dark trials when compared to baseline (light) trials ($t_{25} = -2.37$, $p < 0.03$), the DS's for control and light-dark sessions were not different ($t = -0.34$, ns), indicating that the change in time per arm choice seen in light-dark sessions is no greater than that observed for control sessions (Table 1). This suggests that the decrease in task performance was not due to a generalized change in behavior during dark trials.

Table 1:
Summary of rats' performance (top) and place field (bottom) parameters. Baseline values represent averages of all parameters calculated from the first block of trials during control and light-dark recording sessions. Raw and absolute values of Difference Scores (DS's) are also listed for each parameter. Values represent means ± standard errors. The significant differences from controls ($p < 0.05$) are indicated by bold text.

		DS (Raw Value)		DS (Absolute Value)	
Performance Summary	Baseline Values	Control n = 20 sessions	Light-Dark n= 26 sessions	Control n = 20 sessions	Light-Dark n= 26 sessions
Mean Errors/Trial	0.51 ± 0.09	-0.09 ± 0.15	**-0.49 ± 0.09**	0.50 ± 0.09	0.58 ± 0.07
Mean Time/Arm Choice (sec)	12.36 ± 0.60	0.04 ± 0.03	0.08 ± 0.03	0.08 ± 0.03	0.12 ± 0.03

		DS (Raw Value)		DS (Absolute Value)	
Place Cell Summary	Baseline Values	Control n = 34 cells	Light-Dark n = 38 cells	Control n = 34 cells	Light-Dark n = 38 cells
Mean Firing Rate (Hz)	0.48 ± 0.04	0.02 ± 0.04	0.03 ± 0.05	0.17 ± 0.02	0.21 ± 0.03
Place Field Reliability (%)	63.4 ± 2.51	0.10 ± 0.04	**-0.22 ± 0.05**	0.21 ± 0.03	**0.33 ± 0.04**
Place Field Specificity	4.75 ± 0.19	0.04 ± 0.03	**-0.17 ± 0.03**	0.14 ± 0.02	0.19 ± 0.03
In-Field Firing Rate (Hz)	7.90 ± 1.18	-0.01 ± 0.03	-0.13 ± 0.05[1]	0.16 ± 0.02	**0.26 ± 0.03**
Out-of-Field Firing Rate (Hz)	0.26 ± 0.03	0.02 ± 0.04	**0.14 ± 0.03**	0.15 ± 0.02	0.18 ± 0.03
Place Field Size (pixels)	2022.67 ± 189.07	0.04 ± 0.05	-0.12 ± 0.06[2]	0.22 ± 0.03	**0.33 ± 0.04**
Spatial Correlation (r) - see note	*NA*	***0.49 ± 0.05***	***0.19 ± 0.03***	*NA*	*NA*

Note: Spatial correlation scores listed in DS (Raw Value) columns represent Pearson's r values, not DS's
[1] Marginally Significant Oneway ANOVA: $F(1,71) = 3.57$, $p = 0.06$
[2] Marginally Significant Oneway ANOVA: $F(1,71) = 2.95$, $p = 0.09$

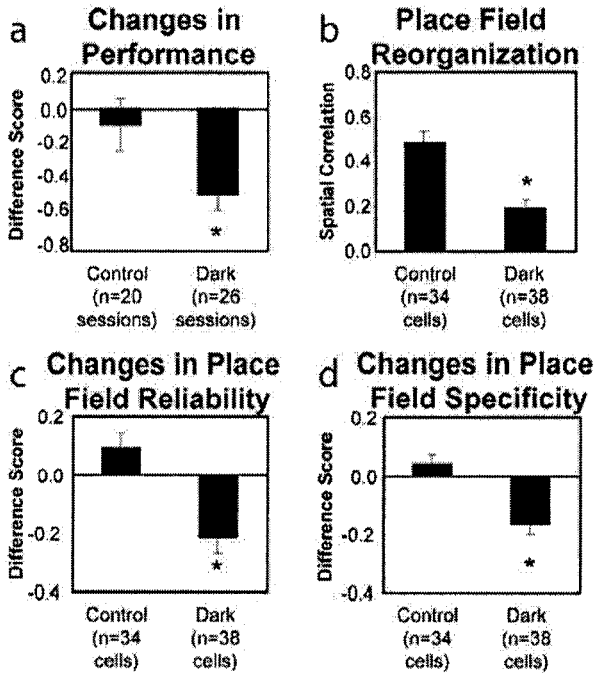

Figure 1.1:
Changes in rats' performance (A), place field reliability (C), and place field specificity (D) are presented as difference scores (DS's) relative to the first block of trials. Rats spatial working memory was significantly worse in dark testing conditions, as reflected in decreased performance difference scores (A). Similarly, darkness caused place fields to reorganize location more (B), and become less reliable (C) and less specific (D). Asterisks indicate significant differences from control manipulations (p's < 0.01).

Next, we evaluated whether darkness significantly affected the mean firing rate, specificity, reliability, spatial distribution of firing, in- and out-of-field firing rates, and place field sizes of place cells. A summary of this analysis can be found in Table 1 and Figure 1.1. We found that there were no significant changes in the mean firing rate of place cells in darkness relative to controls (Raw averages: $F[1, 71] = 0.02$, ns; Absolute Values: $F[1, 71] = 1.0$, ns). The spatial distributions of place cell firing were affected by changes in the visual environment, as indicated by significantly lower spatial correlation scores (i.e., greater spatial reorganization) during dark trials relative to controls (F[1, 71]

= 27.31, $p < 0.001$, Fig. 1.1b). Place field reliability was significantly decreased in darkness when compared to control conditions ($F[1, 71] = 20.17$, $p < 0.001$, Fig. 1.1b). Similarly, place field specificity was significantly decreased in dark testing conditions when compared to controls ($F[1, 71] = 27.98$, $p < 0.001$, Fig. 1D). Consistent with a decrease in place field specificity, there was a marginally significant decrease in the in-field firing rates ($F[1, 71] = 3.57$, $p = 0.06$), while out-of-field firing rates significantly increased in darkness compared to controls ($F[1, 71] = 6.97$, $p < 0.02$). In addition, darkness was associated with a marginally significant decrease in place field size ($F[1, 71] = 2.95$, $p = 0.09$). It should be noted that, although the raw DS values for in-field firing rate and place field size were not statistically significant, the absolute value of these DS's were (In-Field Firing Rate DS_{abs}: $F[1, 71] = 6.34$, $p < 0.02$; Place Field Size DS_{abs}: $F[1, 71] = 4.93$, $p < 0.04$). Although there was some variability in an individual place cell's response, this indicates that in the majority of cases, place fields became less specific in darkness because the in-field firing rates decreased, out-of-field firing rates increased, and place fields became smaller. Figure 1.2 shows an example of two simultaneously recorded place cells in light and dark testing conditions. Darkness induced a striking change in the location of the place field, as well as a reduction in the reliability and specificity of each cells' place field. Consistent with previous reports (Quirk et al., 1990; Markus et al., 1994), this indicates that place fields can change in multiple ways in darkness. We next evaluated which of these changes is related to the increased errors in dark testing conditions.

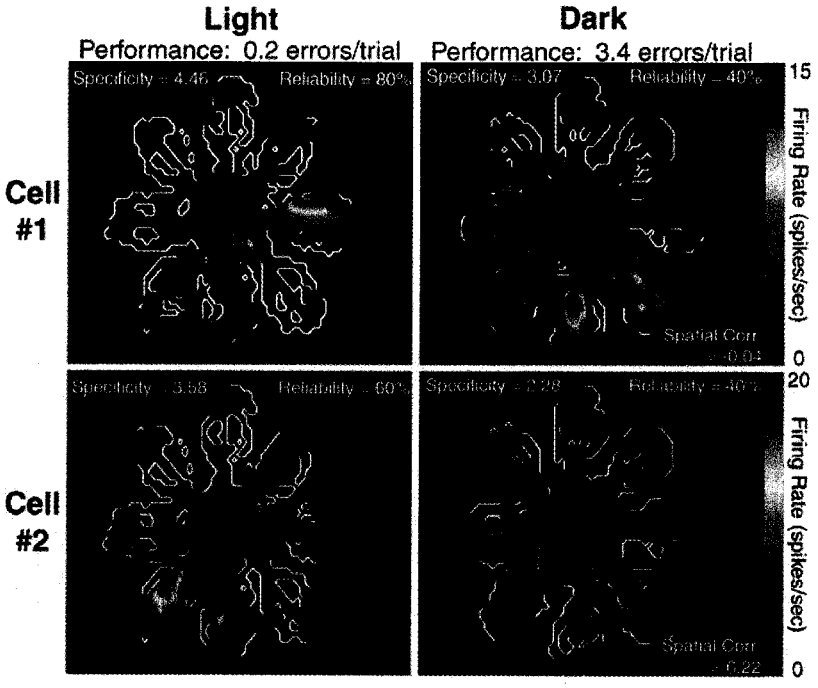

Figure 1.2:
Three-dimensional firing rate maps of two simultaneously recorded place cells in light (left column) and dark (right column) testing conditions. The plots represent the spatial firing patterns of each cell as the rat performed the spatial working memory task. The white outline represents the boundaries of areas on the maze the rat visited. The rat made significantly more errors in darkness (Performance DS = -0.89). Both cells had highly specific and reliable place fields during light trials. Dark testing conditions caused each cell to change their spatial firing patterns in multiple ways. Both cells' place fields reorganized (as indicated by low spatial correlation scores), and became less specific (Specificity DS's: Cell #1 = -0.18, Cell #2 = -0.22) and less reliable (Reliability DS's: Cell #1 = -0.33, Cell #2 = -0.20) in darkness.

Changes in the firing patterns of place cells were correlated with changes in rats' performance of the spatial working memory task by comparing the animal's change in performance with changes in place field specificity, reliability, and degree of reorganization for each light-dark recording session (n = 26). In cases where more than one place cell was recorded simultaneously, an average of the cells' response was

computed. A Pearson's correlation analysis revealed that changes in performance were not significantly correlated with the degree of place field reorganization (r = 0.01, ns, Fig. 1.3a). Similarly, changes in performance were not correlated with changes in place field reliability (r = 0.10, ns, Fig. 3b), in-field firing rate changes (r = -0.06, ns, data not shown), out-of-field firing rate changes (r = -0.19, ns, data not shown) or changes in place field size (r = 0.18, ns, data not shown). In contrast, changes in performance were significantly correlated with changes in place field specificity (r = 0.44, $p < 0.03$, Fig. 1.3c).

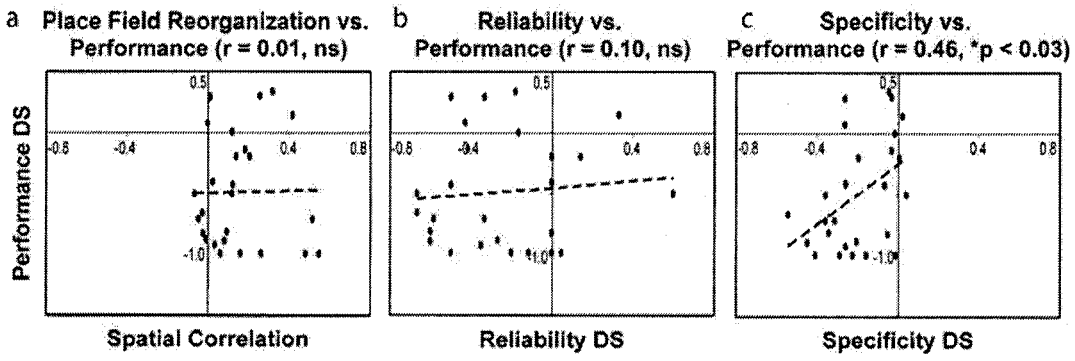

Figure 1.3:
Changes in task performance were assessed for correlations with changes in place field characteristics (r values indicate Pearson's correlation coefficient). Each light-dark recording session with at least one place cell represents one data point (n=26). In cases where more than one place cell was recorded simultaneously, an average response of the place cells was computed. This analysis indicated that the dark-induced changes in task performance are correlated with changes in place field specificity (C), but not changes in place field reliability (B) or the degree of spatial reorganization (A).

DISCUSSION

Although place fields changed in multiple ways when rats perform a spatial working memory task poorly (e.g., reorganization of place fields and reduced place field

specificity and reliability), we found that not all of these variables predicted the degree of task impairment. It was found that only the changes in place field specificity were correlated with changes in task performance. That is, the less specific place fields became in the darkness, the worse the rats performed the spatial working memory task. It appears that the decrease in place field specificity was due to reduced firing rates within the place fields along with an increase in out-of-field firing rates. This result is consistent with the fact that place fields of mice lacking functional CA1 NMDA receptors are less specific (McHugh et al., 1996), a phenomena which might underlie the spatial learning deficits of these mice (Tsien et al., 1996). In the former experiment, place cells of these mice were not recorded during the performance of a hippocampal-dependent task, making it difficult to define a relationship between the place field properties and learning deficits. The results of the present study therefore, provide direct evidence for a relationship between place field specificity and spatial memory.

Similar to the present findings, Markus et. al. (Markus et al., 1994) showed that place fields reorganized and became less specific and reliable in the darkness. In contrast to our results however, they found that, on average, rats that had a higher tendency to make errors (in both light and dark testing conditions) had less reliable place fields, while the specificity of their fields did not correlate with task performance. Important methodological differences may account for this discrepancy. First, the reliability and specificity measures used in the Markus et al. and the current study were computed differently. Markus et al. calculated place field specificity in terms of information content (Skaggs et al., 1993), which reflects how well an individual cell's firing predicts the rat's location. In addition, Markus et al. assessed place field reliability by computing

average spatial correlation scores (as used in the current study to assess place field reorganization) for each pair of trials in light and dark testing conditions. As mentioned in the Markus et al. study, both of these measures are very sensitive to a cell's firing rate, and can yield highly variable results when analyzing low rate cells (such as place cells). In the present study, only cells with place fields on an arm (as opposed to the center of the maze) were included in the analysis. Therefore, the reliability and specificity measures used in the present study were sufficiently powerful to assess these aspects of hippocampal place fields, while avoiding the variability due to low firing rats.

A second methodological difference between the current and Markus et al. studies is the task rats were performing. Markus et al. used a 'forced-choice' eight-arm radial maze task which does not require spatial or working memory, and does not depend on an intact hippocampus. Accordingly, the extent to which the task is hippocampal-dependent can dramatically affect how place cells respond to environmental manipulations (Zinyuk et al., 2000). Therefore, the increased memory demands of the spatial working memory task could also account for the different results of the current study.

Another explanation for the different findings between the Markus et. al. study and this one could be differential inclusion criterion for the analyses. Markus et. al. only included cells that had place fields in both the light and dark testing conditions. Such a selection method could have biased their sample towards place fields that were more stable and more strongly driven by a pattern completion process in the absence of complete visual information (Marr, 1971; McNaughton and Morris, 1987). The analyses in the current study included all cells that had place fields in one or both of the two blocks of trials, and therefore, include cells that maintained, lost, and gained place fields

in darkness. The current study included cells that exhibited all types of changes in order to more accurately describe the alterations in the population representation of the spatial context sent to hippocampal efferent structures, such as the prefrontal cortex (Swanson, 1981; Jay et al., 1989). This was an important consideration, since accurate performance of the task used in this study is thought to depend on hippocampal-prefrontal circuitry (Floresco et al., 1997). Degraded spatial context information sent from hippocampus would impair the working memory functions of prefrontal cortex, thereby impairing performance of the spatial working memory task.

It should be noted here that it has been demonstrated that the spatial organization of place fields can be important for accurate performance of some spatial tasks (O'Keefe and Speakman, 1987; Huxter et al., 2001; Lenck-Santini et al., 2001; Lenck-Santini et al., 2002). However, performance of the tasks used in these studies depended on the organization of the available spatial cues in the environment. That is, rats could use the configuration of the spatial cues in order to navigate to a single goal location. When the cues were rotated (Huxter et al., 2001; Lenck-Santini et al., 2001; Lenck-Santini et al., 2002) or unavailable (O'Keefe and Speakman, 1987), the rat's behavior and spatial organization of its place fields were bound to the rotated cues (Huxter et al., 2001; Lenck-Santini et al., 2001; Lenck-Santini et al., 2002) or the rat's previously established internal representation of the goal location (O'Keefe and Speakman, 1987). Therefore, there appear to be certain conditions in which the spatial organization of place fields is very important for performance of spatial tasks. Since the organization of the spatial cues did not predict the location of a goal in the current study (i.e., rats were required to visit all eight arms regardless of the status of the available spatial cues), the place field

reorganization we observed in darkness could have been the result of place fields realigning to the remaining information rats had available to them during dark trials (i.e., local maze or self-motion cues).

Although we found that rats' spatial working memory was impaired in dark testing conditions, there were recording sessions during which their performance did not change in darkness, despite the fact that highly specific place fields reorganized (see Fig. 3C). Our results are consistent with previous explanations of darkness-induced effects on place field properties (Mizumori et al., 1999a): place fields that persist in dark testing conditions may reflect memories about familiar features of the spatial context. Therefore, the highly specific place cells recorded in sessions in which rats performed well may have been strongly driven by mnemonic inputs about the remembered spatial context, thereby enabling the rat to guide its behavior appropriately in darkness. Alternatively, these cells may have relied on self-motion (Russell et al., 2003) or local environmental (Shapiro et al., 1997; Save et al., 2000) cues that may have been present in darkness. Since rats were performing a well-learned task, the population of active place cells may have been able to reorganize their spatial firing patterns to align to the information available to the rat in the darkness. This could have led to an overall spatially different, yet still highly specific representation of the spatial context that the rat could use to maintain proper hippocampal activity and flexible spatial working memory. Recording sessions in which rats performed poorly in darkness could have been associated with place cells that were not able to integrate non-visual information to develop specific place fields that could be used to guide behavior.

The current study utilized the fact that rats' performance of the spatial working memory task declined in dark testing conditions to test which properties of place fields are important for accurate spatial working memory. Darkness was associated with significant reorganization of place fields as well as decreases in place field specificity and reliability. Importantly, however, changes in performance were correlated with decreases in place field specificity, and not the overall degree of place field reorganization or decreases in place field reliability. The selectivity of the correlation suggests that, at least in some cases, the specificity of place fields is more directly related to accurate spatial working memory than place field reliability or the degree to which fields reorganize in space. The quality of representation of the spatial context in hippocampus could have impacted the degree to which hippocampal efferent structures, such as the prefrontal cortex, could use spatial context information for working memory computations.

Chapter 3: Ventral Tegmental Area and Context Processing

The ventral tegmental area (VTA) is important for several learning and memory functions. Dopamine release from VTA neurons has been most studied in terms of it's role in reinforcement learning (Schultz, 2002; Wise, 2004; Fields et al., 2007). Several lines of evidence indicate that the activity of putative VTA dopamine neurons may be involved in signaling aspects of a reward prediction error implemented in the process of learning about cues that predict the availability of reward (Hollerman and Schultz, 1998; Bayer and Glimcher, 2005; Tobler et al., 2005; Roesch et al., 2007).

The VTA is also thought to be critical for a variety of other learning and memory functions, most notably spatial learning and working memory (Gasbarri et al., 1996; Hefco et al., 2003). Accordingly, dopamine receptor manipulation has been shown to alter neuronal activity in both the hippocampus (Kentros et al., 2004; Gill and Mizumori, 2006) and prefrontal cortex (Sawaguchi et al., 1988; Williams and Goldman-Rakic, 1995), structures known to be important for spatial learning and working memory, respectively. In addition, dopamine appears to be involved in detecting novel contexts, as evidenced by dopamine release in VTA target areas, such as the NAc, prefrontal cortex, and hippocampus upon exposure to an unfamiliar environment (Ihalainen et al., 1999). This dopamine release, at least in NAc, is dependent on indirect hippocampal inputs to VTA (Legault and Wise, 2001). Novelty-induced hippocampal dopamine release has been shown to facilitate synaptic plasticity mechanisms that presumably underlie the formation of context-specific memories (Li et al., 2003; Lemon and Manahan-Vaughan, 2006). Thus, it has been proposed that the hippocampus serves to detect new information not already represented in long-term memory (Vinogradova, 2001; Mizumori et al.,

2007b), which then may lead to altered VTA dopamine release in hippocampus in order to update long-term memory traces (Lisman and Grace, 2005).

Although VTA and hippocampal circuitry is considered to play an essential role in normal spatial learning, and despite the well known firing properties of VTA (presumably dopamine) neurons during appetitive conditioning, it is not understood how VTA neural activity relates to performance of a hippocampal-dependent, spatial task. Therefore, we first sought to characterize the activity of VTA neurons while rats performed a spatial working memory task. We then tested whether VTA neuronal activity is dependent on similar sorts of context and reward information that hippocampal neurons have been found to be dependent on. We found that the activity of the majority of VTA neurons was related to task related events (i.e., rewards and predictive cues), similar to what has been reported previously in rats performing conditioning tasks (Pan et al., 2005; Roesch et al., 2007). In addition, testing rats in a spatially extended environment revealed for the first time that the activity of the majority of VTA neurons was also modulated by several aspects of the movements made to obtain rewards. Furthermore, reward-related activity was gated by contextual information, while movement related activity was not. Therefore, different components of VTA neural codes can be independently regulated.

METHODS

Subjects

Male Long-Evans rats (4-6 months old) were obtained from Simonson Labs (Gilroy, CA). All rats were housed individually in Plexiglas cages in a temperature and humidity-controlled environment and were maintained on a 12 hour light/dark cycle. All

experiments were conducted during the light portion of the cycle. Food and water were provided *ad libitum* for 5 days as rats acclimated to the colony room prior to being handled daily and reduced to 85% of *ad libitum* feeding weights. All rats had unlimited access to water throughout the experiment. All animal care and use was conducted according to University of Washington's Institutional Animal Care and Use Committee guidelines.

Surgical procedures and histology

Details concerning the construction of recording stereotrodes and microdrives and surgical procedures can be found in previous reports (Puryear et al., 2006; Smith and Mizumori, 2006a). Briefly, stereotrodes were constructed by twisting together two 25 μm laquer-coated tungsten wires (California Fine Wire, Grover Beach, CA) and passing them through a 30 ga stainless steel guide cannula. Four stereotrodes were then secured to each microdrive (one per hemisphere) with epoxy. Stereotrodes were cut with sharp surgical scissors to leave 2-3 mm of each stereotrode exposed at the tips of the guide canulae and were electroplated (AgCl solution, Fisher Scientific, Pittsburgh, PA) as necessary to obtain a final impedance of 200-400 kΩ (tested at 1 kHz). Rats were anesthetized with isofluorane (5% mix with O_2 for induction with 1-4% for maintenance of anesthesia) and given an antibiotic (Batryl; 5 mg/kg) and an analgesic (Ketofen; 5 mg/kg). The microdrive assemblies were implanted dorsal to VTA according to the following coordinates relative to bregma: -5.25 mm posterior, 0.7 mm lateral, 7 mm ventral (Swanson, 1998). A reference electrode (114 μm Teflon coated stainless steel wire) was implanted near the corpus collosum and a ground screw was implanted into the

skull. Rats were then given one week of free feeding to fully recover from surgery before being placed back on food-restriction to begin recording experiments.

The final position of each stereotrode was marked by passing a 25 μA current through each recording wire for 25 sec while rats were under 5% isofluorane anesthesia. Rats were then given an overdose of sodium pentobarbital and transcardially perfused with a 0.9% buffered saline solution, followed by 10% formalin. The electrodes were then retracted and the brain was removed and allowed to sink in a 30% sucrose-formalin solution. Forty-micron coronal sections were sliced through the midbrain with a cryostat. The sections were then stained with cresyl violet and recording locations were verified by comparing depth measurements and reconstruction of the electrode tracts. Only cells determined to be located in VTA (Swanson, 1998) were considered for data analysis.

Single-unit recording

Prior to each session, rats were connected to the recording equipment by a pre-amplification headstage, which was equipped with a pair of infrared diode arrays to track the animal's movements and directional heading. All stereotrodes were checked daily for spontaneous neural activity. If no clear neural activity was encountered stereotrodes were lowered in approximately 25 μm increments (up to 175 μm per day) until clear, isolatable units were observed. The animal's position and electrophysiological data were recorded on a Neuralynx Cheetah data acquisition system (Neuralynx, Inc., Bozeman, MT). The position of the animal was monitored by an infrared video camera mounted on the ceiling above the maze (sampled at 30 Hz). Multiunit activity was recorded simultaneously and independently on each wire of the stereotrode. Incoming signals were amplified (1,000-10,000 times), filtered between 600 Hz and 6 kHz and passed

through a window discriminator that triggered a 2 msec sampling period (at 16 kHz) when an impulse from either channel passed a user-defined threshold.

Single units were isolated from the multiunit records using standard cluster-cutting software (MClust; A.D. Redish, University of Wisconsin). In addition, a template-matching algorithm (written by Chris Higginson) was used offline to facilitate separation of unique spike waveforms. To ensure a high degree of unit separation quality, we only included cells with a signal-to-noise ratio of at least 3:1, exhibited stable clusters throughout the recording session, and exhibited a clear refractory period in the inter-spike interval histogram following cluster cutting.

Differential-reward spatial working memory task

Rats were habituated to the testing environment by allowing them to freely forage for chocolate milk on an eight-arm radial maze for 30 minutes a day until they reliably drank the chocolate milk and continuously moved about the maze for the full 30 minutes. Rats were then trained to perform a differential reward, win-shift spatial working memory task using procedures reported previously (Pratt and Mizumori, 2001). Briefly, the end of each arm was baited prior to the start of each trial with either a large (5 drops) or small (1 drop) amount of chocolate milk on alternating arms. Maze arms containing large or small amounts of reward (counterbalanced across rats) were held constant for each rat throughout training. Each trial consisted of a Study and Test Phase. The Study Phase started with the individual and sequential presentation of four of the eight arms (two large and two small reward arms, randomly selected for each trial) in which the rat ran down to the end of each arm and consumed the chocolate milk reward. After presentation of the fourth arm, the Test Phase began by allowing the rat access to all

maze arms. The rat was then required to collect the remaining rewards by choosing the four arms that were not presented during the Study phase. The trial ended once all eight arms were visited and the rat returned to the center of the maze, where it was confined for a 2 minute inter-trial interval before a new trial started. Entries into previously visited arms were classified as errors. Once the rat performed 15 trials in approximately one hour for seven consecutive days, recording electrodes were surgically implanted into VTA.

In order to test the context-dependency of task performance and VTA unit activity, we employed a within-subjects design in which each recording session consisted of two blocks of five trials. During the first block of trials (Baseline trials), rats performed the task with the extra-maze cues and rewards in their familiar configuration (i.e., the configuration present during initial training). Following completion of the 5th trial, rats performed a second block of five trials under one of the following four context conditions; either 1) the same conditions as the Baseline trials (Control), 2) the maze room lights extinguished (Darkness), thereby eliminating the visuo-spatial cues in the maze environment, 3) the locations of the large and small rewards switched (Reward Location Switch), or 4) two rewards (1 large and 1 small reward, randomly chosen) were omitted from the study phase of each trial (Reward Omission). Although the context manipulation performed on a given day of testing was chosen randomly, care was taken to insure that adequate numbers of cells were recorded for each type of manipulation.

Data analysis

Task performance: As mentioned above, animals performed the differential-reward, spatial working memory task under varying contextual conditions. In order to test

whether altering contextual information had any impact on task performance, we calculated the average number of errors in the first and second blocks of trials (E_1 and E_2, respectively), and computed a Performance Difference Score (DS_P) according to the following formula:

$$DS_P = \frac{(E_1 - E_2)}{(E_1 + E_2)}$$

In this case, negative DS_P values indicate that the animal made more errors in the second block of trials, whereas positive DS_P values indicate that the animal made fewer errors in the second block of trials. Average DS_P values were compared across contextual manipulations by means of a oneway ANOVA, with Bonferroni *post hoc* tests ($\alpha = 0.05$). All statistical tests were performed using SPSS 13.0 (Chicago, Il).

We were also able to asses the subject's discrimination of, and preference for large and small reward locations by calculating the probability of choosing a large reward arm during the first four arm choices of the test phase for each trial. Since subjects were food restricted during this study, it was expected that they would retrieve the large rewards before the small rewards when given the choice during the test phase of the trial. Therefore, we assessed whether there was a significant Spearman's correlation ($\alpha = 0.05$) between the first four arm choices and the probability that the choice would be a large reward arm for each block of trials.

Baseline firing properties: Previous studies in rats have delineated putative DA from non-dopamine neurons in VTA on the basis of waveform duration, average firing rate, burst firing characteristics (i.e. burst rate, non-burst rate, per-cent spikes in bursts, and intra-burst firing rate), and firing rate changes in response to pharmacological

manipulations (Robinson et al., 2004; Pan et al., 2005; Roesch et al., 2007). We calculated these basic electrophysiological properties for neurons in this study according to these previously established methods. It was of interest to determine whether alterations of the context had any major impact on these basic firing properties. Therefore, we computed Difference Scores (DS) for average firing rate, non-burst firing rate, burst rate, percent spikes in burst, and intra-burst firing rate for each block of trials according to the following formula:

$$DS_x = \frac{(x_2 - x_1)}{(x_1 + x_2)}$$

In this case, positive and negative DSs indicate an increase and decrease, respectively, in the variable in the second block of trials. Each of these DSs were submitted to oneway ANOVAs and Bonferroni *post hoc* tests ($\alpha = 0.05$) to determine whether there was significant contextual modulation of these variables.

As with previous studies, pharmacological tests with quinpirole (D2 receptor agonist) and eticlopride (D2 receptor antagonist) were initially incorporated to aid in discriminating between dopamine and non-dopamine neurons. However, due to very inconsistent task performance on days following drug treatments, it was necessary to discontinue this method. In contrast to substantia nigra *pars compacta* (SNc), recent evidence has indicated that there does not seem to be clear electrophysiological or pharmacological criteria for determining the DA content of VTA neurons (Margolis et al., 2006). This is likely due to the heterogeneous neurotransmitter content in VTA neurons relative to SNc (Yamaguchi et al., 2007; Nair-Roberts et al., 2008b). Nevertheless, an additional 13 VTA neurons were tested for their response to quinpirole

(0.4 mg/kg, s.c.) at the conclusion of the experiment, and their firing rates for 10 min before and 20 min following injection were calculated. Four neurons exhibited a >50% reduction in firing rate, and were considered putative dopamine neurons (Pan et al., 2005). However, the spike characteristics (waveform duration and firing rate) did not conform to the commonly used criterion for classifying putative dopamine neurons (Fig. 2c). Although it is important to determine how putative dopamine neurons respond during spatial working memory tasks, we did not restrict our analyses to this cell type. Rather, we sought to assess whether the population of VTA neurons, as a whole, exhibit significant event-related firing during performance of the spatial working memory task and whether these responses were gated by contextual information.

Reward-related activity: Due to previously established reward-related activity of rodent VTA neurons (Hyland et al., 2002; Pan et al., 2005; Roesch et al., 2007) we were interested in evaluating whether similar activity occurred during performance of the spatial working memory task. To do this, the rewards were located in small metal cups mounted to the end of each maze arm, which served as 'lick-detectors' and were connected to the recording equipment (custom designed by Neuralynx, Inc.). An event marker was automatically inserted into the data stream when the rat licked the cup, providing an instantaneous measurement of the time the rat first obtained the reward. Peri-event time histograms (PETHs) were then constructed (50 msec bins, ± 2.5 s around each reward event) for all reward events, as well as separate PETHs for large and small reward events. A cell was considered to have a significant excitatory reward response if it passed the following two criteria: 1) the cell was observed to have a peak firing rate within ± 150 ms of reward acquisition and 2) the peak rate was >150% of it's average

firing rate for the block of trials. These criteria were applied to PETHs collapsed across reward amounts, and separately for large and small reward events.

In order to determine if reward responses were dependent on contextual information, we analyzed cells that exhibited a significant reward response in one of the two blocks of trials. First, we calculated a Reward Activity value (*RA*) which was the average firing rate in the ± 150 ms around the time of reward acquisition, expressed as a per-cent change relative to the cell's average firing rate for each block of trials. In order to directly compare the reward-related activity for each context manipulation, these values were normalized to the maximum value observed for each manipulation, yielding a normalized *RA* value for the first and second block of trials (RA_{n1} and RA_{n2}, respectively). The same calculations were performed for non-rewarded arms in order to address whether similar reward prediction errors observed in putative dopamine neurons (Hollerman and Schultz, 1998; Tobler et al., 2003; Roesch et al., 2007) occur during performance of this spatial working memory task. We then created scatter plots of RA_n's for each block of trials. The theory behind this method is that, if VTA reward-related activity was independent of context information, RA_{n1} and RA_{n2} should be similar in each block of trials, thereby lining up near the diagonal of the scatter plots. Therefore, we devised a *Reward Activity Change Index* (*RACI*), which calculated the distance of each data point to the diagonal line according to the following formula:

$$RACI = \frac{\sqrt{2(RA_{n1} - RA_{n2})^2}}{2}$$

Average *RACI* values were then compared across context manipulations with a oneway ANOVA and Bonferroni *posthoc* tests ($\alpha = 0.05$). This analysis was carried out for

reward responses collapsed across large and small reward amounts as well as for responses at large rewards. Unit responses at small rewards were not analyzed separately due to the paucity of VTA neurons that only exhibited small reward-related responses (see Results).

Cue-related activity: Rodent VTA neurons have also been shown to fire in response to cues that predict the availability of rewards (Pan et al., 2005; Roesch et al., 2007). During the course of these recordings, it was noticed that some neurons consistently fired when the first arm of each trial was presented (i.e., the 'first arm' cue signaling the start of the trial and availability of reward). Therefore, we began to manually insert event markers, online, into the data stream, indicating the time in which the first arm of each trial was made available. For this subset of cells, PETH's were also constructed (100 ms bins, ±2.5 s around each cue event time). A cell was considered to have a significant excitatory cue response if it passed the following two criteria: 1) the cell was observed to have a peak firing rate within ±150 ms of cue presentation and 2) the peak rate was >400% of it's average firing rate for the block of trials. It was necessary to use a more conservative set of criteria since VTA neurons typically fired at lower rates during the inter-trial interval, which led to spurious firing that would have led to false identification of cue-related responses with less stringent criteria. Due to the fact that these cue-related responses were unexpectedly observed, we recorded a relatively small number of these types of neurons (see Results). Therefore, we were unable to determine if these responses were modulated by contextual information.

Movement-related activity: We sought to determine if the rodent VTA also contained neurons that were sensitive to particular movements the rat made during performance of

the spatial working memory task. The most reliable behaviors to assess were times in which the rat made 180° turns at the ends of maze arms to return to the center maze and make another arm choice. Therefore, event markers were manually inserted, offline via inspection of the positional data record, to indicate the time in which those movements started (i.e., Turn movements). We also marked times in which the rat started its forward movement towards the center of the maze (Inbound movements) and assessed movement-related activity on outbound journeys by utilizing the PETHs constructed around reward acquisition events. We then constructed PETHs around these events (100 ms bins, ±2.5 s around the event). Average firing rates were calculated 1000 ms before reward events, and 1000 ms after Turn and Inbound events to determine whether the cell exhibited an elevated firing rate (greater than 50% it's average firing rate) during outbound movement, turns, or inbound movement, respectively. Five VTA neurons were omitted from this analysis due to very low firing rates (< 1.0 spike/s). Due to the relatively few VTA neurons that exhibited significant responses during one or more of those behaviors, we were not able to determine whether they were sensitive to changes in contextual information.

In addition to firing relative to particular movements displayed by freely moving animals, we sought to determine if the firing rates of VTA neurons were also correlated with these aspects of the animal's movement. As described previously (Yeshenko et al., 2004; Gill and Mizumori, 2006), we assessed each neuron's firing rate for a significant Pearson's linear correlation with velocity or acceleration ($p < 0.05$). In order to avoid contamination with any reward-related activity (i.e., when the animal was not moving)

and to ensure adequate sampling of these movement parameters, we limited the ranges of velocity and acceleration to 3-30 cm/s and 3-30 cm/s^2, respectively.

For cells with significant firing rate correlations with velocity and/or acceleration during one or more blocks of trials, we assessed whether these movement parameters were influenced by contextual information. Similar to the *RACI* values calculated for analysis of reward-related activity, we calculated an *r Value Change Index* (*RVCI*) by substituting the velocity and acceleration correlation r values for the RA_n values used in the reward response analysis. Significant contextual effects on *RVCI* values were also assessed by means of oneway ANOVAs and Bonferroni *posthoc* tests ($\alpha = 0.05$).

RESULTS

Task Performance: Spatial Working Memory

VTA neural activity was recorded while rats performed two blocks of five trials of a differential reward, spatial working memory task on an eight-arm radial maze under varying context conditions (Pratt and Mizumori, 2001; Puryear et al., 2006) (see Methods and Fig. 2.1a).

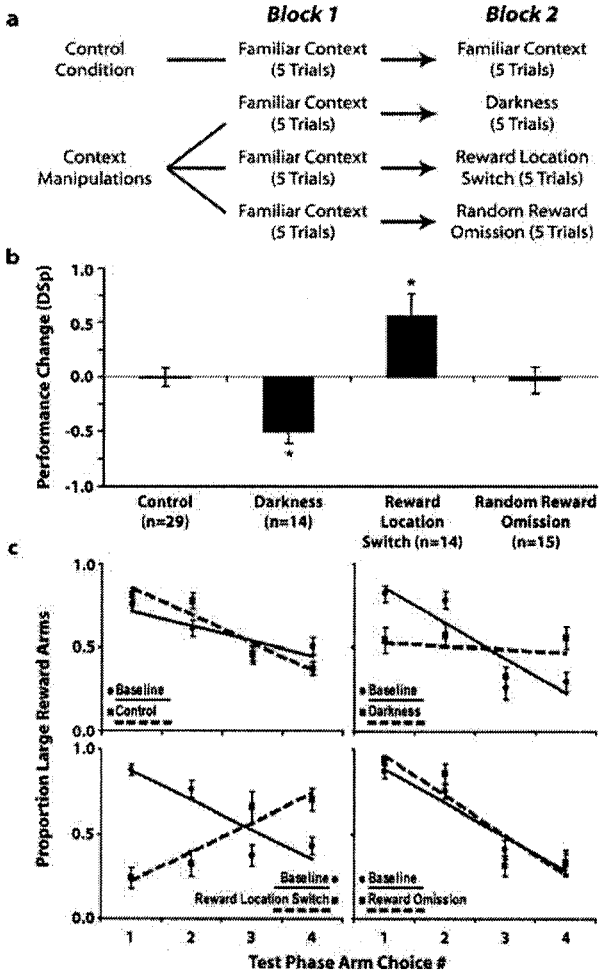

Figure 2.1:
Experimental design and task performance. **(a)** Rats performed the differential reward, spatial working memory task under varying contextual conditions. Each day rats would first perform 5 baseline trials (Block 1) under familiar contextual conditions (i.e., the same as they had been trained under). Rats would then perform another 5 trials (Block 2) under the same contextual conditions (Control), with the lights extinguished (Darkness), with the locations of large and small rewards switched (Reward Location Switch), or with two rewards omitted from each trial (Random Reward Omission). Each of these testing conditions altered some aspect of the context (i.e., visuo-spatial information, locations of rewards, and reward probability, respectively). **(b)** Rats' spatial working memory ability was affected changes in contextual information. Displayed is the change in the average number of committed in Block 2 relative to Block 1. Positive and negative values indicate a decrease and increase, respectively, in errors in Block 2. Asterisks indicate significant differences (oneway ANOVA, $p < 0.05$). **(c)** Rats' ability to discriminate locations of large and small rewards depended on contextual information. Displayed is the probability of choosing an arm containing a large reward for the first four arm choices during the test phases. Error bars represent s.e.m.

The average number of errors per trial committed by the rats was similar in the first block of trials for each testing condition ($F[3,74] = 1.32$, $p < 0.2$, Fig. 2.2). Therefore, we determined whether alterations of the context produced changes in spatial working memory abilities by calculating the difference in the average number of errors across blocks of trials (see Methods). A oneway ANOVA revealed a significant main effect of context ($F[3,74] = 12.45$, $p < 0.001$), indicating that alterations of the context changed the rats ability to perform the spatial working memory task (Fig. 2.1b). Consistent with previous reports (Puryear et al., 2006), task performance was significantly degraded in the Darkness compared to Control conditions ($p < 0.02$), indicating that rats made more errors when performing the task without the aid of visuo-spatial cues. Interestingly, performance was significantly enhanced for Reward Location Switch manipulations ($p < 0.002$), indicating that rats made fewer errors after the locations of large and small rewards were changed. On the other hand, Reward Omission manipulations did not alter task performance relative to Control manipulations ($p = 1.0$).

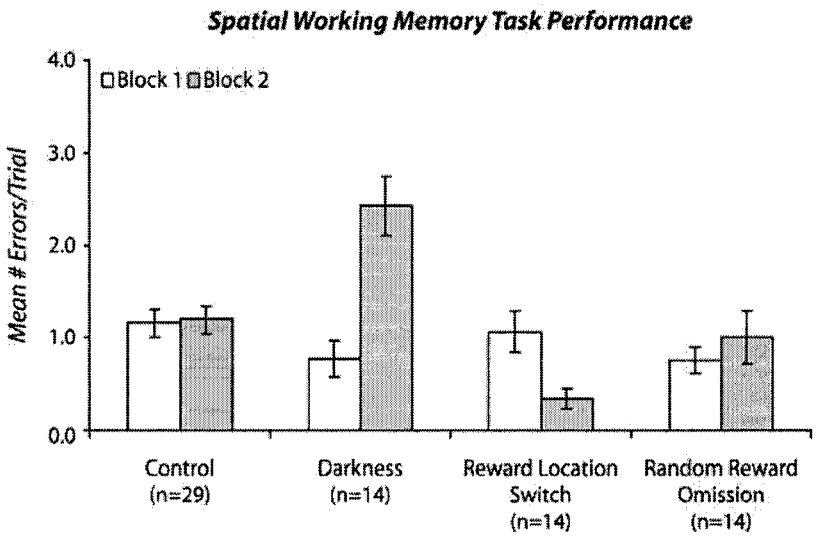

Figure 2.2:
Spatial working memory task performance. The average number of errors made during each trial are plotted relative to different context conditions. There were no differences in performance during Block 1 across context conditions (oneway ANOVA, $p > 0.05$). However, rats made more errors in dark testing conditions and fewer errors when the locations of large and small rewards were switched.

Task Performance: Reward Preference

Subjects demonstrated their ability to discriminate the locations of large and small reward during the first block of trials by preferentially selecting arms associated with large rewards (Fig. 2.1c). For Control sessions, there were significant negative correlations for both blocks of trials between the first four arm choices during the test phase and the probability that the arm chosen was a large reward arm (Block 1: $r = -0.38$, $p < 0.001$, Block 2: $r = -0.61$, $p < 0.001$). Importantly, this demonstrates that, under constant context conditions, rats' preference for large rewards remained stable across both blocks of trials. This discrimination, however, was influenced by alterations of contextual information. When tested in Darkness, rats were unable to differentiate large and small reward arms, as evidenced by a loss of correlation between arm choice number

and the probability of choosing a large reward arm (Block 1: $r = -0.70$, $p < 0.001$, Block 2: $r = -0.09$, $p > 0.5$). Despite the fact that Reward Location Switch manipulations caused rats to perform the spatial working memory task better, they were unable to adjust to the changes in the reward locations. This was manifested by a significant negative correlation between arm choice number and the probability of a large reward arm choice in the first block of trials (Block 1: $r = -0.71$, $p < 0.001$) along with a significant positive correlation (Block 2: $r = 0.57$, $p < 0.001$) between arm choice number and the probability of a large reward arm choice in the second block of trials (i.e., the first and second arm choices tended to be arms that contained large rewards in Block 1). Similar to control conditions, Reward Omission manipulations did not alter rat's preference for large rewards (Block 1: $r = -0.76$, $p < 0.001$, Block 2: $r = -0.75$, $p < 0.001$).

Histology and Spike Characteristics

We recorded a total of 89 neurons that were determined to be located in VTA (Fig. 2.3a). As has been done in previous studies in freely behaving rodents (Robinson et al., 2004; Pan et al., 2005; Roesch et al., 2007), we attempted to identify putative dopamine neurons in our sample population by testing their responses to the administration of a D2 agonist (quinpirole; 0.4 mg/kg, sc). However, neurons that decreased firing rates following quinpirole administration did not always conform to the predominant criteria of putative dopamine neurons based on spike characteristics (i.e., spike duration > 1.5 ms and average firing rate < 10 spikes/s, see Fig. 2.3b & c). Although spike duration may be due to differences in filter settings used across studies, these data favor the proposition that these standard criteria may not always be a reliable way of identifying putative dopamine neurons in VTA (Margolis et al., 2006; Marinelli et

al., 2006). This is especially the case for recordings in (food deprived) freely moving rodents, which do not allow for labeling of individual neurons in order to confirm the neurotransmitter content (i.e., presence of tyrosine hydroxylase, TH). However, we found that there was a clear delineation of VTA cell populations based on their average firing rate (Fig. 2.3b). Therefore, we will refer here to VTA neurons as either low rate (average firing rate < 10 spikes/s) or high rate (average firing rate > 10 spikes/sec) cells.

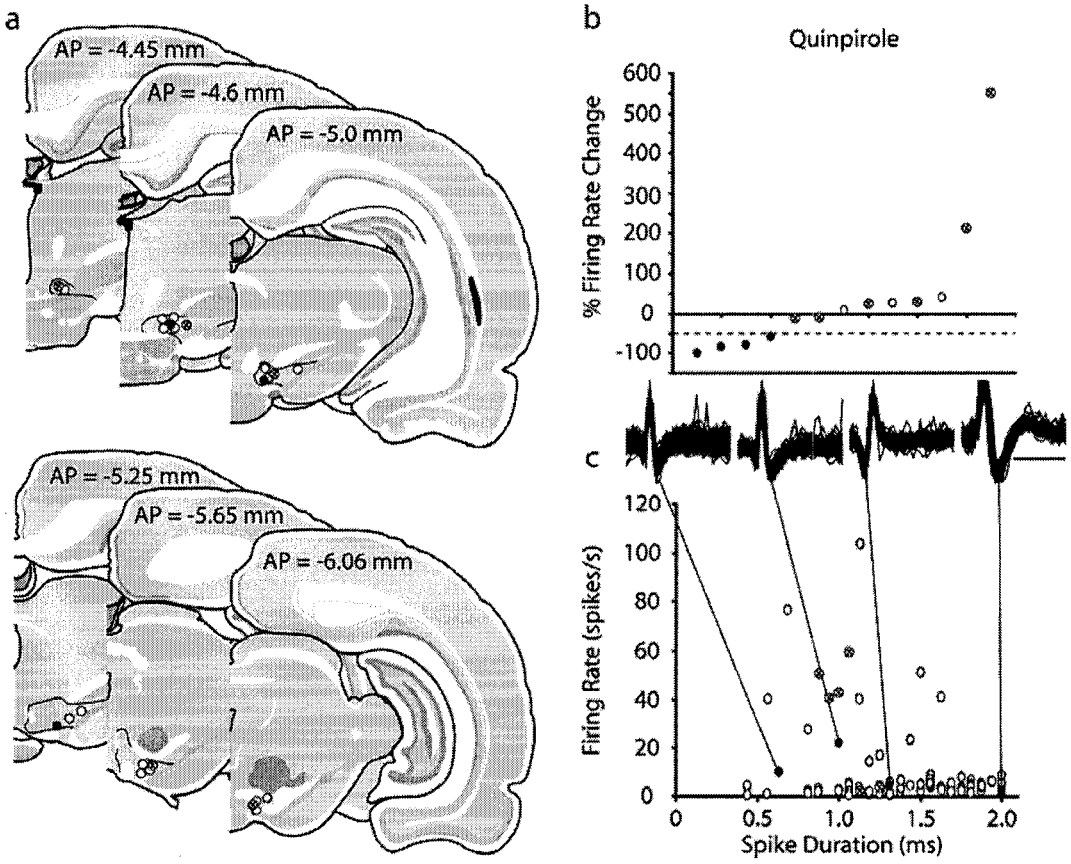

Figure 2.3:
Locations and identification of VTA neurons. **(a)** Distribution of neurons localized to VTA; each dot may represent the location of more than one neuron. **(b)** Pharmacological tests on a subset of VTA neurons. A neuron was considered a putative dopaminergic neuron if the firing rate decreased by more than 50% (dotted line) following administration of the D2 agonist, quinpirole (0.4 mg/kg, sc). **(c)** Comparison of average firing rates and spike durations of VTA neurons indicated a clear delineation of two populations of neurons based on average firing rate (high rate neurons, >10 spikes/s; low rate neurons, < 10 spikes/s). Note that putative dopamine neurons (sample waveforms depicted above, scale bar = 1 ms) did not conform to the commonly used classification criteria. Black dots indicate putative dopamine neurons; crossed dots indicate neurons that exhibited strong theta rhythmic activity.

Firing profiles of VTA neurons

The firing characteristics of VTA units (i.e., spike duration, average firing rate) as well as the burst firing characteristics (non-burst firing rate, burst rate, percent spikes in

burst, and intra-burst firing rate) were similar to what has been reported previously in freely behaving rats (Lee et al., 2001; Hyland et al., 2002). Of note, VTA neurons that displayed significant task- and behavior-related firing patterns (described below) exhibited a broad and overlapping range or spike widths, average firing rates, and burst-firing characteristics (Fig. 2.4).

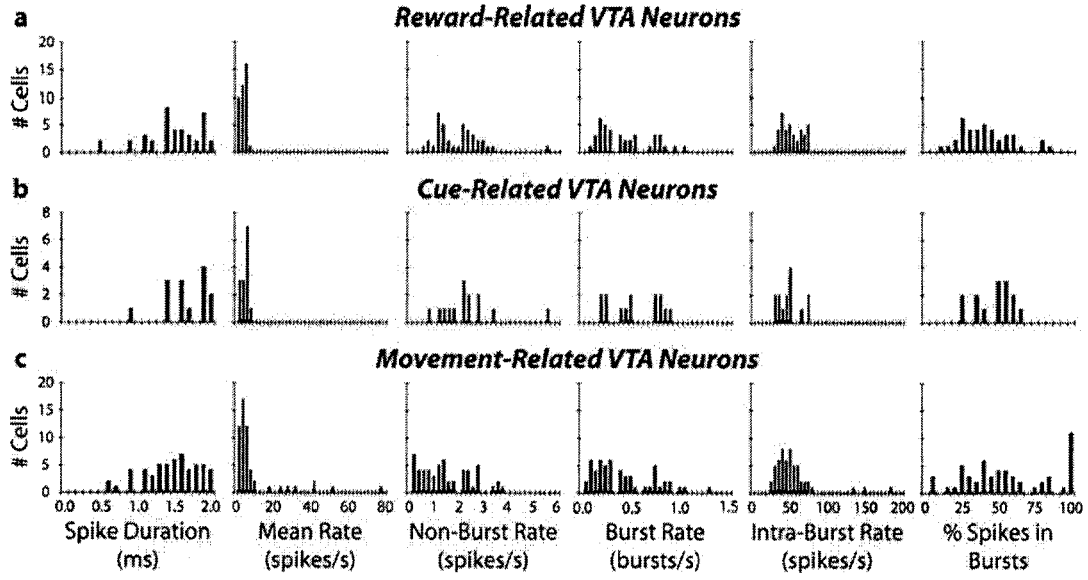

Figure 2.4:
Distribution of spike and firing properties of (a) reward-, (b) cue-, and (c) movement-related neurons. The population of cells that exhibited each type of task- and behavior-related consisted of cells with very similar distributions of spike durations, average firing rates, non-burst rates, burst rates, intra-burst firing rates, and % spikes within bursts. However, the population of movement-related neurons contained some high rate and high bursting neurons.

None of these basic spike parameters were significantly altered following changes to the context (Fig. 2.5). What follows is a description of the most prominent task-related and behavioral correlates of VTA neuronal firing as rats performed the spatial working memory task. For each type of neural-behavioral correlate, the baseline properties are

described first, followed by a description of the context-dependency of this neural activity.

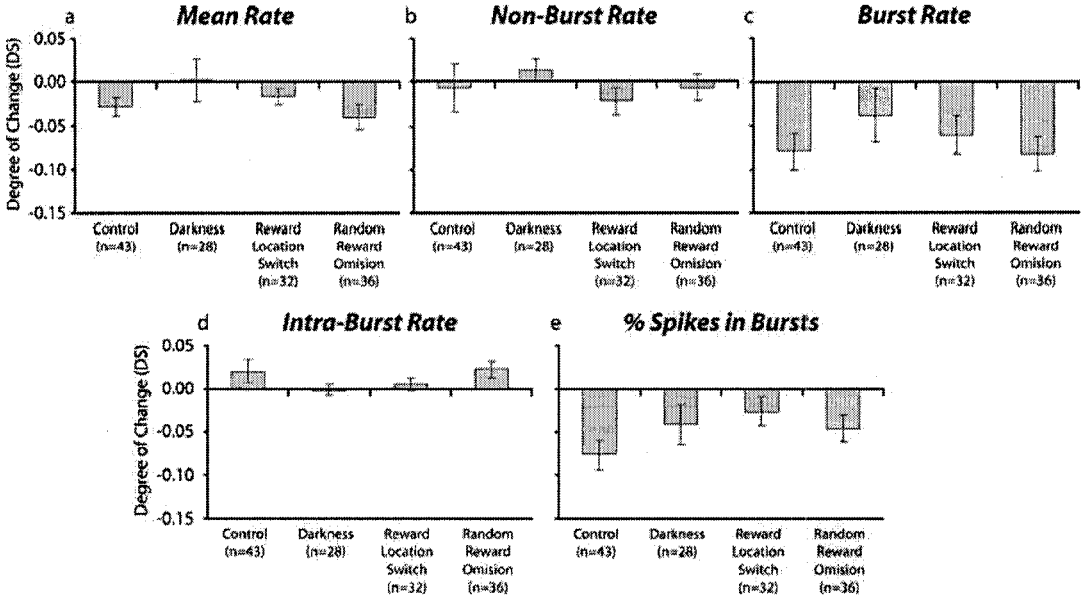

Figure 2.5:
Contextual information does not influence the basic firing properties of VTA neurons. There were no significant changes in mean firing rates **(a)**, non-burst firing rate **(b)**, burst rate **(c)**, intra-burst firing rate **(d)**, and % spikes in bursts **(e)** across context conditions (oneway ANOVA, p's > 0.05).

Reward-Related Responses

Baseline: Of the 89 cells, 5 were excluded from this analysis due to very low firing rates (< 1 spike/sec). Consistent with previous reports in rodents (Hyland et al., 2002; Pan et al., 2005; Roesch et al., 2007), we observed a large population of VTA neurons (49%, 41/84) that exhibited short-latency, excitatory responses upon acquiring rewards (see Methods for classification criteria and Fig. 2.6).

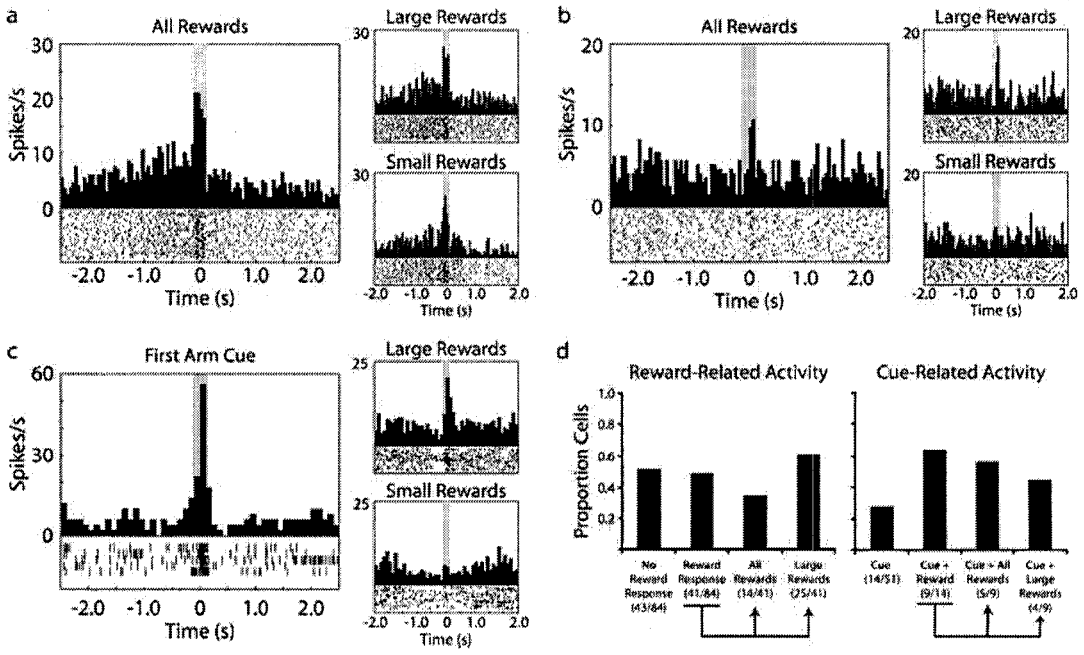

Figure 2.6:
Peri-event time histogram examples of reward- and cue-related activity of VTA neurons. **(a)** An example of a neuron that exhibited a short-latency, excitatory response upon acquisition of rewards (t_0, bin width = 50 ms). Left histogram represents neural activity relative to acquisition of all rewards. Top and bottom histograms on the right represent neural activity relative to acquisition of large or small amounts of reward, respectively. The activity of this neuron did not discriminate reward amounts. **(b)** An example of a neuron that preferentially fired upon acquisition of large rewards. Left histogram shows only a modest excitatory response when considering all rewards together. However, top and bottom right histograms show that the reward-related firing occurred upon acquisition of large and not small rewards. **(c)** Histogram on left shows an example of a neuron that exhibited a significant excitatory response when the first arm of each trial was made available (t_0, bin width = 100 ms). Top and bottom histograms on the right indicate that this neuron also exhibited significant reward-related upon acquisition of only large rewards. **(d)** Population summary of the proportion of VTA neurons that demonstrated significant reward- and cue-related activity, as well as the proportion of neurons that encoded the conjunction of rewards and cues. **(a-c)** Grey shaded time ranges indicate time periods analyzed for significant increases in firing rate.

Overall, 49% of neurons (41/84) exhibited a significant reward response when considering large and small rewards together. Depicted in Figure 2.6a is an example of one neuron that exhibited excitatory responses at both large and small reward encounters.

Consideration of large and small rewards separately indicated that 34% (14/41) of cells responded to both large and small rewards, whereas 61% (25/41) of cells only responded to acquisition of large rewards. No cells were found to selectively fire relative to acquisition of small rewards (see Fig 2.6c for summary). The cell depicted in Figure 4b only exhibited a significant response upon acquisition of large rewards. All reward-related cells exhibited low average firing rates (> 10 spikes/s). Cells that responded to both reward amounts fired at an average rate of 3.54 ± 0.35 spikes/s (mean ± sem) and exhibited waveform durations of 1.41 ± 0.10 ms. Cells that responded to only large rewards had similar average firing rates and spike durations (3.41 ± 0.30 spikes/s and 1.48 ± 0.08 ms, respectively). A oneway ANOVA indicated that there were no significant differences in firing rates or spike durations of cells responding to all rewards and cells responding to only large rewards (Mean Rate: $F[1,38] = 0.29$, $p > 0.50$; Spike Duration: $F[1,38] = 0.79$, $p > 0.70$).

Context-Dependent Reward Activity: For neurons that exhibited significant reward-related activity in one or more blocks of trials, we determined whether the activity was gated by contextual information by computing the change in firing rates in the 150 ms period around the time of reward acquisition across blocks of trials (see Methods and Fig. 2.7).

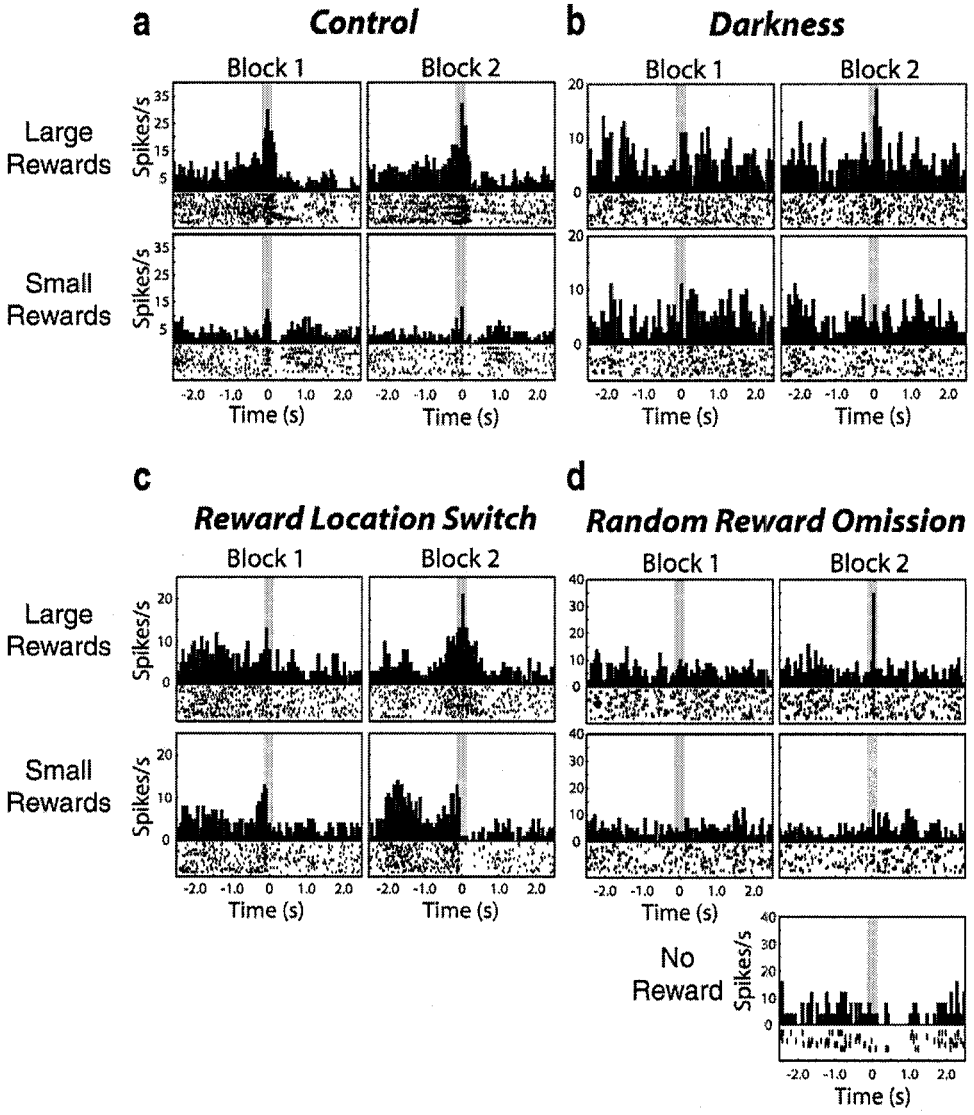

Figure 2.7:
Contextual control of reward-related activity. **(a-d)** Peri-event time histograms of representative neurons recorded under varying contextual conditions. Shaded regions are as in **Fig. 2.6**. **(a)** Example of a neuron that exhibited a robust response upon acquisition of large rewards which was unaltered in Control conditions. **(b)** Example of a neuron that did not exhibit reward-related activity in Block 1, but developed a significant response to large rewards in dark testing conditions (Block 2). **(c)** Example of a cell that developed a significant response to large rewards in Block 2 when they were switched to locations that previously contained small rewards. **(d)** Example of a neuron that did not exhibit any reward-related activity in Block 1, but developed a significant response upon acquisition of large rewards after rewards had been randomly omitted in Block 2). Note that the activity of this cell was inhibited when the rat did not obtain an expected reward (lower right histogram).

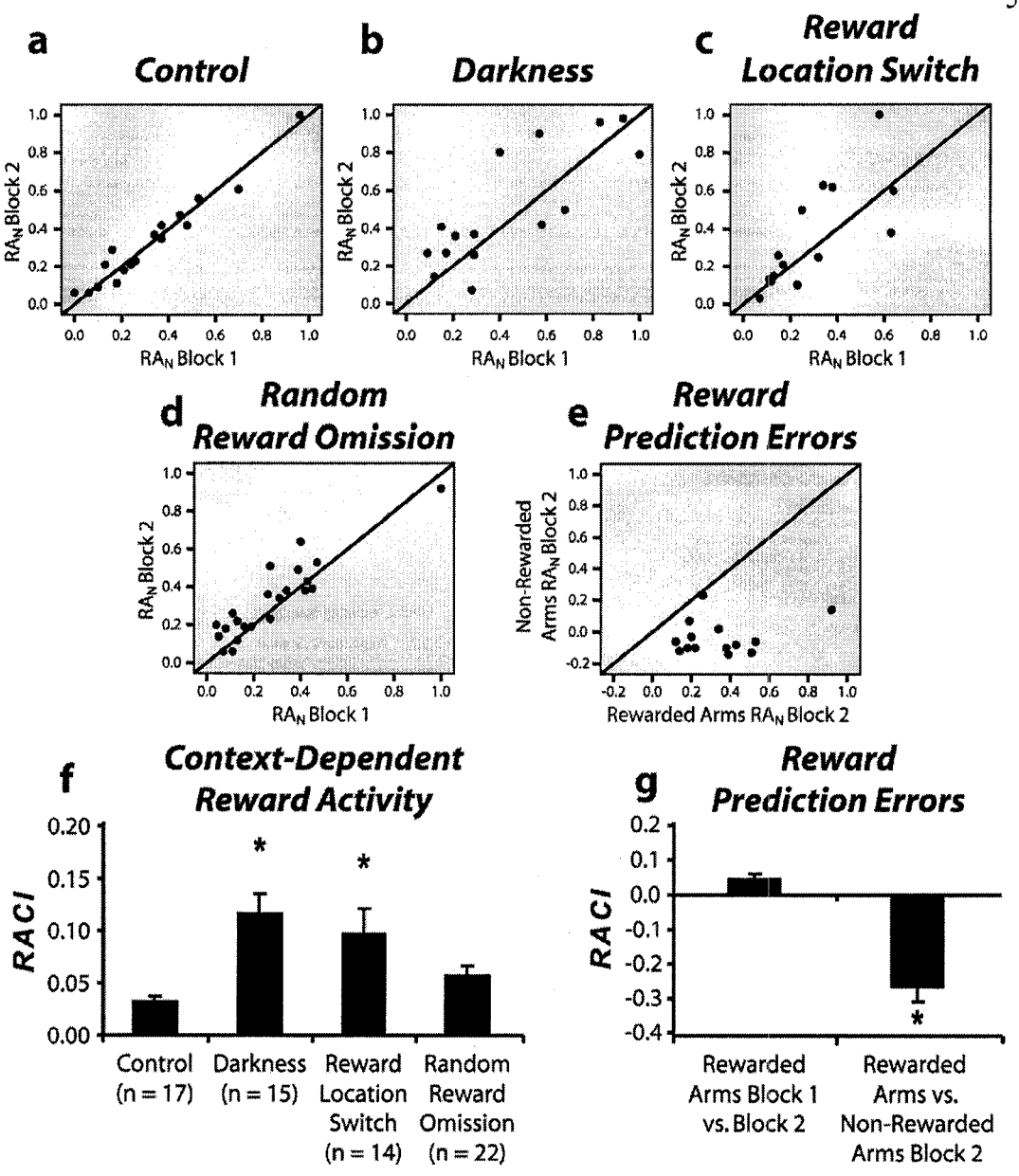

Figure 2.8:
Population summary of contextual control of reward-related activity. **(a-d)** Plotted is each neuron's normalized reward activity (RA_N, defined in Methods) for each block of trials during each contextual condition. **(e)** Plotted are RA_N values for rewarded and non-rewarded arms in Block 2 (x and y axes, respectively). **(f)** Average changes in reward activity (RACI, defined in Methods) for neurons recorded in each testing condition. Asterisks indicate significant differences from Control ($p < 0.05$). **(g)** RACI values with direction of change taken into account (i.e., increased or decreased activity in Block 2 relative to Block 1) for Random Reward Omission Conditions. Asterisk indicates a significant difference across comparisons ($p < 0.05$). Error bars indicate s.e.m.

A oneway ANOVA comparing an index of changes in reward activity (i.e., *RACI*, defined in Methods; larger RACI values correspond to larger changes in reward-related firing) for all (large and small) rewards revealed a significant main effect of context conditions ($F[3,67] = 6.10$, $p < 0.002$, Fig. 2.8f). Bonferroni *post hoc* tests revealed that *RACI* values were significantly larger for Darkness ($p < 0.003$) and Reward Location Switch ($p < 0.04$) manipulations compared to Control conditions. The mean *RACI* values for cells recorded in the Reward Omission condition were not different from Controls ($p = 1.00$). A similar result was obtained when only considering reward activity at large rewards. In this case, there was a significant main effect of context manipulation ($F[3,73] = 4.14$, $p < 0.01$, Fig. 2.9), in which *RACI* values were significantly higher for Reward Location Switch compared to Control conditions ($p < 0.01$). Although there was an increase in *RACI* values for Darkness and Reward Omission manipulations, these increases were not significantly different from Control conditions ($p = 0.16$ and $p = 1.0$, respectively). Overall, these analyses indicate that the reward-related activity of VTA neurons is, at least in part, bound to information about the current context.

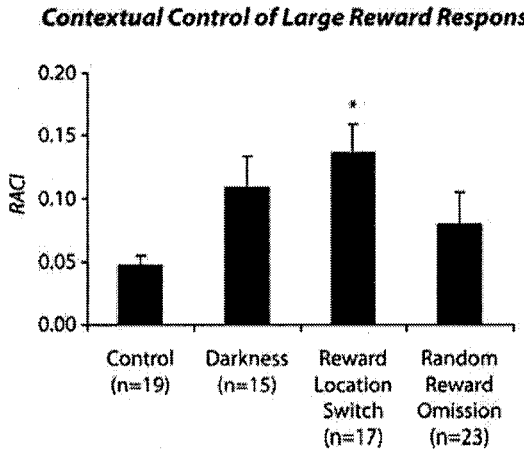

Figure 2.9:
Contextual control of large reward responses. Average changes in large reward activity (RACI, defined in Methods) for neurons recorded in each testing condition. The asterisk indicates that changing reward locations induced significant alterations of large reward responses when compared to Control conditions (oneway ANOVA, $p < 0.05$). Although RACI values for Darkness and Random Reward Omission conditions were increased, they did not reach statistical significance.

Reward Prediction Errors: The Random Reward Omission manipulation enabled us to determine whether the reward prediction errors that have been well described for putative dopamine neurons recorded in conditioning paradigms (Hollerman and Schultz, 1998; Tobler et al., 2003; Roesch et al., 2007) also occur during performance of a spatial working memory task. We compared average *RACI* values for comparisons between rewarded and non-rewarded arms in Block 2 to *RACI* values for comparisons between rewarded arms across Blocks 1 and 2. For this analysis, directionality of the *RACI* values was taken into consideration in order to discriminate between increases and decreases in firing rates. A Paired Samples t-Test revealed a significant decrease in *RACI* values for the comparison of rewarded and non-rewarded arms ($t_{13} = 7.92$, $p < 0.001$, Fig. 2.8e & g), indicating that there was a significant degree of neural inhibition when rats did not find the reward they expected. Importantly, this analysis indicates that negative reward

prediction error signals, similar to those demonstrated during performance of conditioning tasks (Hollerman and Schultz, 1998; Bayer and Glimcher, 2005; Pan et al., 2005; Roesch et al., 2007), also occur in VTA neurons during performance of more complex and natural forms of learning.

Cue-Related Responses

Baseline: We tested a total of 54 VTA neurons for significant responses when the first arm of each trial was presented to the animal (i.e., the first "cue" signaling the start of the trial and availability of reward). Of these cells, 3 were omitted from this analysis due to very low average firing rates (< 1 spike/sec). Overall, 27% of cells tested (14/51) exhibited significant excitatory responses when the first arm was made available (Fig. 4c). As with reward-related neurons, all cue-responsive cells fired at low average rates (3.99 ± 0.40 spikes/s) and had waveform durations of 1.61 ± 0.08 ms. Interestingly, 64% (9/14) of cue-related cells also exhibited significant reward-related responses (see Fig. 2.6c for example). Fifty-six percent of these cells (5/9) only fired relative to large rewards, while 44% (4/9) responded to both large and small rewards (Fig. 2.6d). This analysis indicates that a large proportion of cue-responsive VTA neurons also exhibited some type of reward-related activity. Due to the relatively small sample size, we were unable to determine whether the cue-related responses were dependent on contextual information.

Movement-Related Responses

Baseline: To investigate the potential influences movement variables could have on the firing properties of VTA neurons, we determined whether the firing rates of VTA cells correlated with the velocity and/or acceleration of the animal. Overall, 66% of cells

(59/89) exhibited significant firing rate correlations with either the velocity or acceleration of movement (see Fig. 2.10a-d for examples). Of these cells, 39% (23/59) of the cells' firing rates were correlated with velocity only, 22% (13/59) with acceleration only, and 39% (23/59) with both velocity and acceleration (Fig. 2.9e).

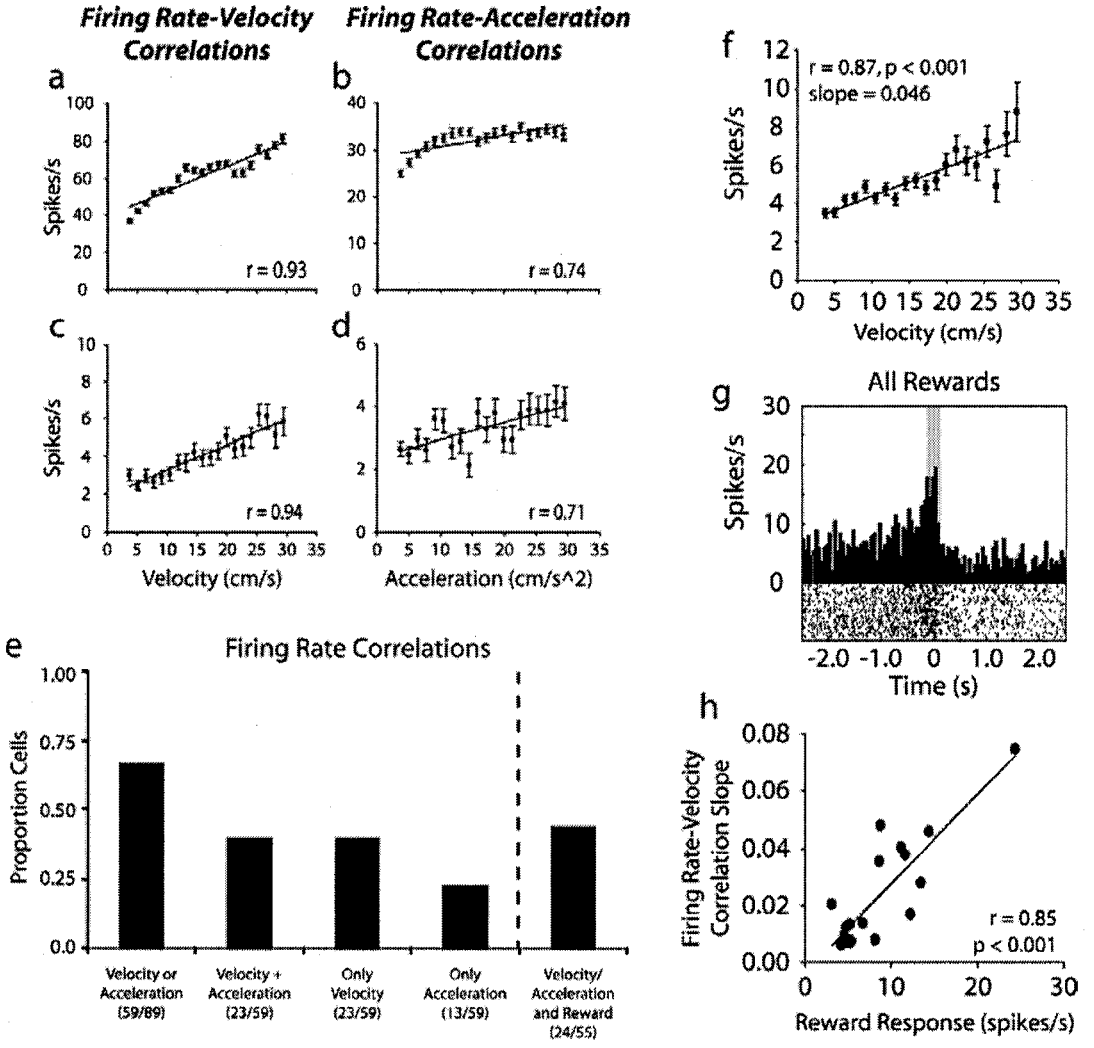

Figure 2.10:
Velocity and acceleration modulation of VTA neuronal activity. **(a & b)** Examples of two high rate neurons whose firing rates correlated with the velocity **(a)** and acceleration **(b)** of the rat (Pearson's r value and linear regression line included in each plot, $\alpha = 0.05$). **(c & d)** Examples of two low rate neurons whose firing rates correlated with the velocity **(c)** and acceleration **(d)** of the rat. **(e)** Population summary of the proportion of neurons that exhibited significant firing rate correlations with velocity and/or acceleration. Plotted to the right of the dotted line is the proportion of velocity- or acceleration-modulated neurons that also exhibited significant reward-related activity. An example of one neuron that exhibitited a dual code of movement and reward is plotted in **f** and **g**. **(h)** For neurons that exhibited movement- and –reward related activity, the degree to which its firing rate was influenced by changes in velocity (i.e., the slope of the linear regression lines, plotted in **a-d & f**) was significantly correlated with the magnitude of response upon acquisition of reward.

Although some neurons with significant modulation by velocity or acceleration exhibited high average firing rates, most cells in the distribution had average firing rates similar to neurons that displayed reward- and cue-related firing (see Fig. 2.4c), indicating that movement sensitivity of VTA neurons is not restricted to only one cell type, such as high rate GABAergic neurons (Lee et al., 2001). However, we found a systematic relationship between the average firing rate of the cell and the degree to which its firing rate was modulated by changes in velocity or acceleration (i.e., the slope of the firing rate-velocity/acceleration correlation regression line): The higher the average firing rate of the cell, the more sensitive it was to changes in velocity or acceleration (Fig. 2.11).

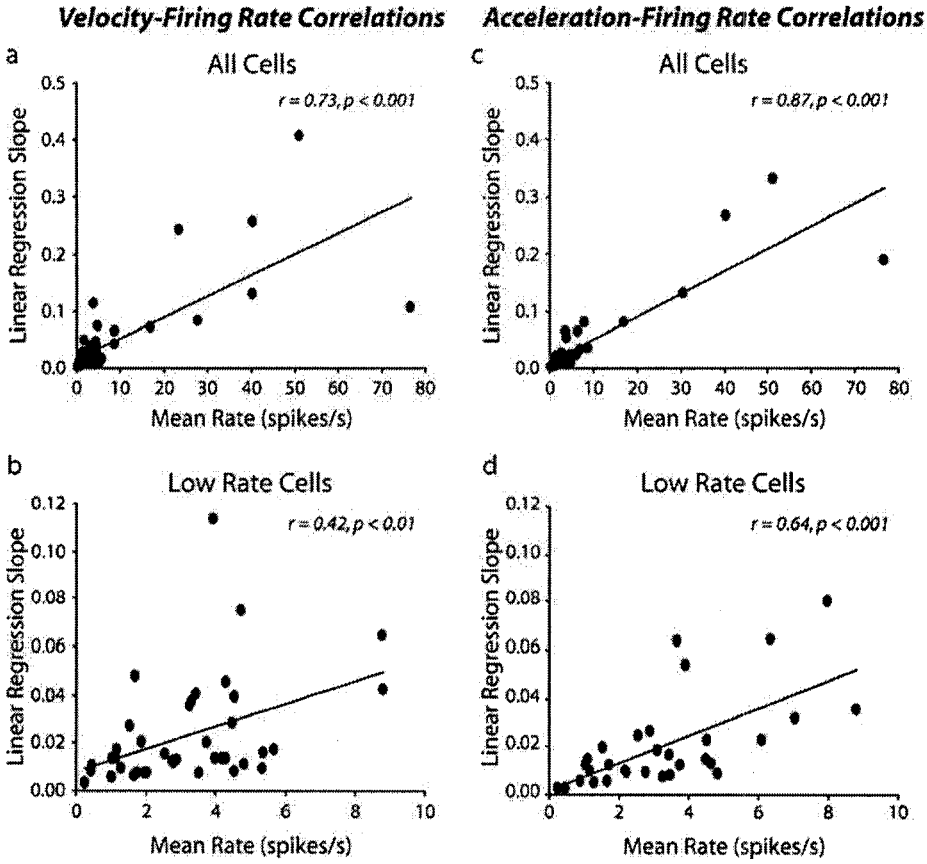

Figure 2.11:
Firing rate-dependent velocity- and acceleration-modulation of VTA neuronal activity. The degree to which the firing rate of a VTA neuron is affected by changes in velocity (i.e., the slope of the firing rate-velocity correlation regression lines) was found to be significantly correlated with the average firing rate of the cell (Pearson's, $\alpha = 0.05$). This was true when considering the entire population of VTA neurons **(a)** and when only considering low rate neurons **(b)**. The same relationship was found for the degree to which the firing rate of a VTA neuron was affected by changes in acceleration when considering all cells **(c)** and only low rate cells **(d)**.

In addition, 44% of VTA cells that exhibited significant firing rate correlations with velocity or acceleration (24/55, after removal of 4 very low rate neurons) also exhibited reward-related firing (Fig. 2.10e). In order to assess whether the velocity or acceleration of the animal could be an important factor in determining the magnitude of reward-related activity of these neurons, we correlated the r values and linear regression

slopes of the correlations between firing rate and velocity/acceleration with the firing rate exhibited upon acquisition of rewards. This analysis revealed that the degree to which the firing rate of the cell is sensitive to changes in velocity was a significant predictor of the magnitude of response upon acquisition of reward (Fig. 2.10h). To our knowledge, this is the first report of movement-dependent responses of reward-related VTA neurons in the freely behaving rodent, indicating that VTA neurons may encode both the acquisition of reward and some aspect of the movements made to obtain it. Furthermore, it appears that the velocity of the animal may be an important factor, or cue, that determines the magnitude of reward response for this population of VTA neurons.

Context-Independent Movement Activity: To establish whether the neural coding of movement information is context-dependent, we determined whether the relationship between firing rate and movement measurements was consistent across context manipulations.

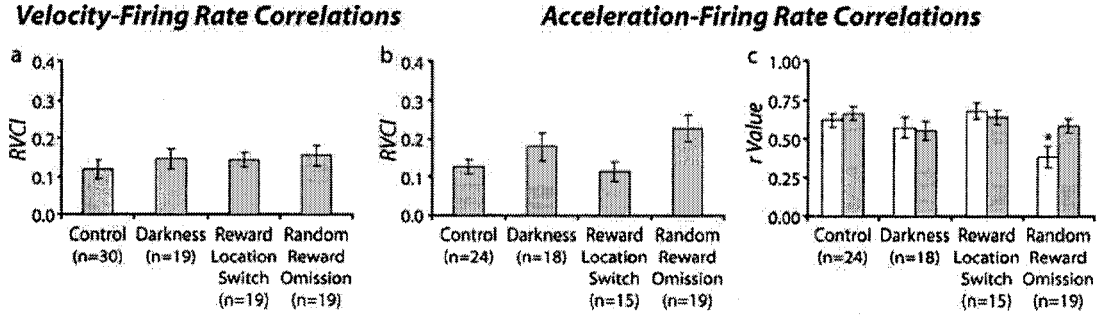

Figure 2.12:
Contextual information does not influence movement-related neuronal activity. Average changes in the r values (RVCI, defined in Methods) for cells with firing rate correlations with velocity **(a)** and **(b)** acceleration for each contextual condition. Alterations of the context did not induce any changes in the degree to which firing rates were correlated with velocity or acceleration relative to Control conditions. **(c)** Average firing rate-acceleration correlation r values for each block of trials for each contextual condition. Asterisk indicates a significant difference from Control and Reward Location Switch Block 1 values (oneway ANOVA, $p < 0.05$). Error bars indicate s.e.m.

A oneway ANOVA comparing changes in the r values of the firing rate correlations with velocity and/or acceleration (i.e., *RVCI's*, defined in Methods) revealed significant differences across contextual conditions for cells with firing rate correlations with acceleration ($F[3,75] = 3.09$, $p < 0.03$, Fig. 12b), but not velocity ($F[3,89] = 0.47$, $p > 0.70$, Fig. 2.12a). Bonferroni *post hoc* tests on the apparent contextual modulation of acceleration-sensitive cells revealed only marginally significant differences when Random Reward Omission conditions were compared to Control and Reward Location Switch conditions (each $p = 0.07$). Further analysis revealed, however, that the average r values of the acceleration-firing rate correlations were significantly different during the first block of trials across manipulations ($F[3,75] = 4.64$, $p < 0.01$). Bonferroni *post hoc* tests indicated that the correlation r values were significantly lower in the Random Reward Omission condition when compared to Control and Reward Location Switch conditions ($p < 0.03$ and $p < 0.01$, respectively) in the first block of trials. This indicates that the population of cells recorded during Random Reward Omission conditions happened to have had weaker relationships with acceleration during the first block of trials, and that relationship became stronger in the second block of trials (Fig. 2.12c). Therefore, the apparent context-dependency of the relationship between firing rate and acceleration was solely due to differences in the population of cells sampled across context conditions, and not due to the change in context information (i.e., reward probability) per se. Overall, this suggests that any movement information that may be encoded by, or impact VTA firing patterns is not overtly dependent on contextual information.

The firing rates of a small population of VTA neurons (11/84, 13%) were found to increase while the rat was engaging in particular behaviors during performance of the task (see Methods, Fig. 2.13).

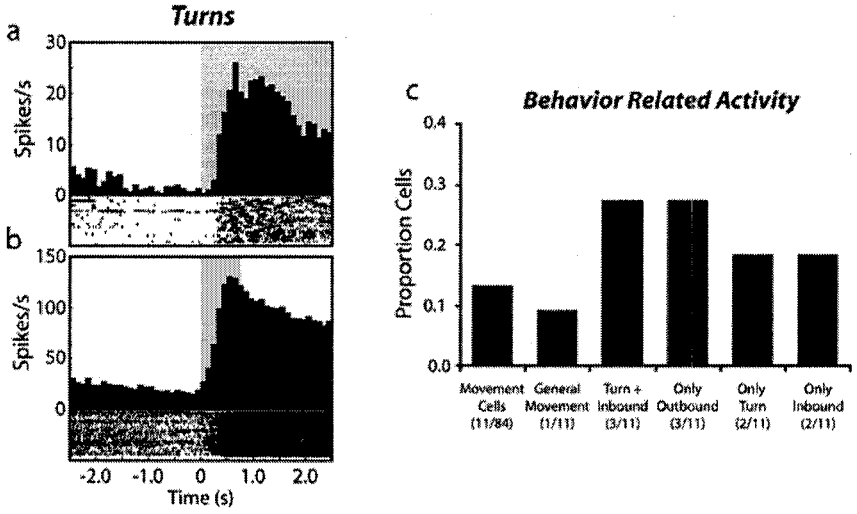

Figure 2.13:
Behavioral correlates of VTA unit activity. **(a)** An example of a VTA neuron that increased firing rate shortly after the onset of turns at the ends of maze arms (t_0, grey shading), prior to traveling towards the center of the maze. **(b)** An example of a VTA neuron that increased firing rate shortly after the onset of turns at the ends of maze arms (t_0, grey shading), and continued to fire at a high rate as the rat traveled towards the center of the maze. **(c)** Population summary of VTA neurons that exhibited prominent activity related to specific behaviors executed on the maze.

Of these, most cells only fired during specific behavioral acts, such as approaching the reward (3/11), turning around at the end of the arm (2/11, Fig. 2.13a), or forward movement toward the center of the maze (2/11). Three cells began firing when the rat started to turn around at the end of the arm, and continued to fire during its forward movement toward the center of the maze (Fig. 2.13b). The remaining cell was found to increase firing rate during all three behavioral periods, suggesting it was sensitive to the general movement state of the animal. Thus, there appears to be rather

sparse coding of behavioral acts in VTA. Due to the relatively few cells exhibiting these types of firing patterns, it was not possible to determine if they were sensitive to changes in contextual information.

It is also noteworthy that a small population of high rate VTA neurons (n = 10) exhibited strikingly rhythmic firing patterns. The firing rates of these neurons were modulated by the ongoing, 4-12 Hz theta rhythm, as evidenced by strong rhythmic patterns in the autocorrelograms and interspike interval histograms (Fig. 2.14). Four of these theta-modulated cells were recorded during performance of the spatial working task. Of these, the firing rates of two cells were modulated by the faster, 7-12 Hz Type-1 (Kramis et al., 1975) theta during periods of forward movement on the maze, very similar to the activity of hippocampal theta cells (McNaughton et al., 1983a; Mizumori et al., 1990) (for example see Fig. 2.14a). The onset of a slower, 4-7 Hz Type-2 theta (Kramis et al., 1975) modulation of firing rates of the other two coincided with the acquisition of rewards (for an example see Fig. 2.14d). Although the small number of theta cells recorded in this study prohibited the ability to determine clearly whether the activity of these cells was sensitive to a change in context information, the few context manipulations performed on these cells did not reveal any alterations in the rhythmic firing patterns (data not shown).

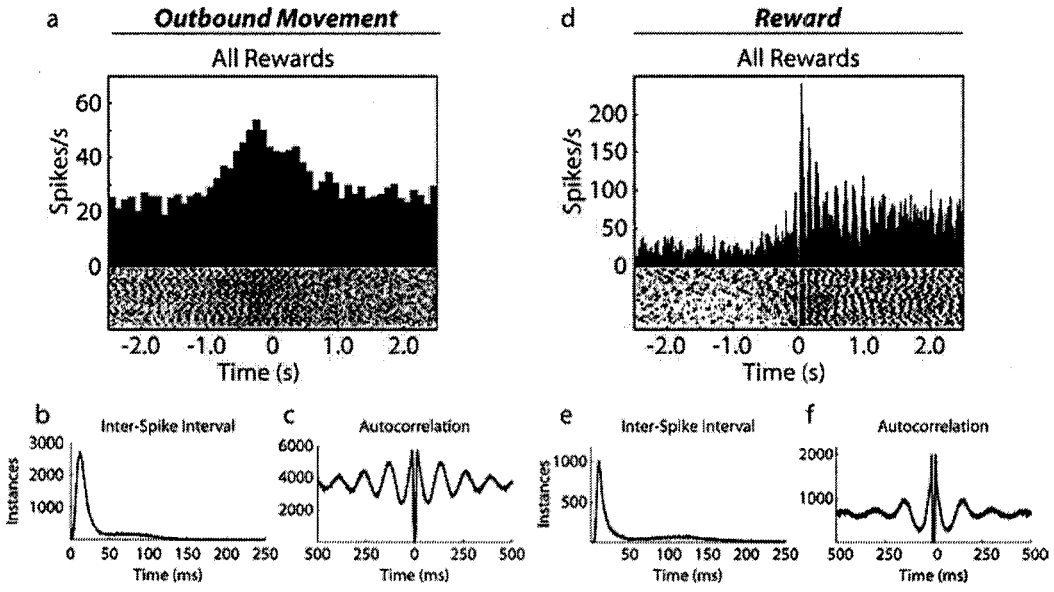

Figure 2.14:
Theta-modulated VTA neuronal activity. The firing rates of 10 VTA neurons were found to be modulated by the ongoing local theta EEG rhythm. Four of these cells were recorded during performance of the spatial working memory task. **(a)** An example of one theta cell that increased firing rate while the rat was walking toward the reward and peaked before the rat acquired rewards (t_0; bin width = 100 ms). The firing rate of this cell was strongly modulated by Type-1 theta activity (7-12 Hz) evident in the inter-spike interval **(b)** and autocorrelation **(c)** histograms. **(d)** An example of one theta cell that was found to dramatically increase firing rate upon acquisition of reward (t_0; bin width = 10 ms to emphasize the strong rhythmic activity), at which point the activity became entrained with the theta rhythm. The firing rate of this cell was strongly modulated by Type-2 theta activity (4-7 Hz) evident in the inter-spike interval **(e)** and autocorrelation **(f)** histograms. Six VTA theta cells were tested for their responses to quinpirole administration. Of these, two were found to dramatically increase their firing rates (> 200%), while the firing rates of the remaining four did not exhibit appreciable change (see **Fig. 2.3b**).

DISCUSSION

Previous experiments have focused on the role of VTA in reinforcement learning using variations of conditioning paradigms. This study characterized VTA neural activity as rats performed a spatial working memory task. We demonstrate that many of the basic components of VTA neuronal activity exhibited during conditioning experiments (i.e.,

reward magnitude- and cue-related activity, reward prediction errors) also exist during goal-directed navigation. Furthermore, we discovered that the activity of the majority of VTA neurons is not only modulated by reward, but also several aspects of the movements made to obtain rewards. Interestingly, we found that contextual information selectively modulated the reward-related activity of VTA neurons. Therefore, different components of VTA neural codes (i.e., reward and movement) appear to be independently regulated. These findings, particularly the conjunction of movement and reward coding, may inform theoretical and computational models of the role of VTA in reinforcement learning (Montague et al., 1996; Lisman and Otmakhova, 2001; Suri, 2002) such that they extend to more complex forms of learning, and aid in the development of adaptive artificial robotic systems (Kawato and Samejima, 2007).

Identity of VTA neurons

Recent evidence suggests that the traditional criteria for identifying putative dopamine neurons are reliable for recordings of substantia nigra *pars compacta* (SNc), but not VTA neurons (Margolis et al., 2006). This is highlighted by the recent finding that 45% of VTA neurons that exhibit quinpirole-induced inhibition of firing (a widely used hallmark of dopamine neurons) are actually not dopamine neurons (Margolis et al., 2006). The apparent discrepancy between VTA and SNc is likely due to the fact that VTA is a more heterogeneous structure and contains a substantial population of glutamatergic neurons (Yamaguchi et al., 2007; Nair-Roberts et al., 2008b). In addition, it appears that some neurons in VTA co-express TH and glutamic acid decarboxylase (an enzyme involved in the synthesis of GABA) (Klink et al., 2001; Olson and Nestler, 2007). This indicates that a subpopulation of VTA dopamine neurons may co-release

GABA, suggesting further functional heterogeneity within the population of VTA dopamine neurons. Until these issues are resolved and reliable methods of identifying the neurotransmitter content of neurons in the freely moving animal are developed, it is prudent to investigate and report on all cell types recorded within VTA. In this way a more complete understanding of the roles of VTA in learning and memory functions may be achieved.

Reward and movement responses of VTA neurons

The key reward properties of putative VTA dopamine neurons were replicated here with the use of a more complex cognitive task. Importantly, this study also highlights novel aspects of VTA unit activity because of the particular behavioral and mnemonic demands of the spatial working memory task. Most notably, it has been reported that reward-related activity diminishes after animals learn to predict rewards (Schultz, 2002). Since the recordings in the current study were performed in well trained animals that were able to predict when and where rewards would be obtained, one might have expected a paucity of reward-related activity. The large proportion of reward-related neurons found in this study suggests that there are other factors that influence the degree of reward activity. Since each trial presents a unique order of locations that had to be remembered in order to perform the task well, one possibility is that reward information needed to be placed within a continually changing temporal and spatial context in order for neurons to predict reward. Thus the inherently less predictable nature of rewards in our spatial working memory tasks may have led to continued observation of reward-related firing of VTA neurons. Nevertheless, significantly decreased activity was observed when expected rewards were omitted, suggesting that negative reward

prediction error signals also occur during performance of spatial working memory tasks. Future experiments should study in more detail the potential role reward prediction error signals of VTA neurons play in context-dependent spatial learning.

We show here that movement is a major contributor to the firing patterns of most VTA neurons. High rate neurons in this study, similar to identified GABAergic neurons (Lee et al., 2001), were found to encode general movement states as well as specific behavioral acts, such as turning and forward movements. However, movement encoding was not limited to high rate neurons since a large proportion of low rate neurons were found to be sensitive to aspects of the animal's movement (i.e., velocity or acceleration). Also, there was a systematic relationship between the average firing rate of a cell and the degree to which that same cell was sensitive to movement. This may indicate that individual VTA neurons are equipped with a heterogeneous distribution of membrane ion channels important for fast ion conductance, akin to hippocampal interneurons (Jonas et al., 2004). This distribution may predispose VTA cells to be sensitive to quickly changing variables (i.e., velocity or acceleration) over protracted periods of time. Since 44% of movement-related neurons also exhibited reward-related activity, it appears that many VTA neurons are able to transition to a firing mode characterized by short high frequency bursts, a firing pattern more suited for coding discrete events or stimuli. Thus, VTA neurons seem to switch coding of one type of information to another by passing from one firing mode to another. Furthermore, since the degree of movement sensitivity of a given VTA neuron predicted the magnitude of the reward response, it may be that internal representations of an animal's movement state are coupled to the expected consequences of behavior in VTA.

Context-dependency of VTA neuronal activity

One report in primates suggests that dopamine neurons represent a context-dependent reward prediction error (Nakahara et al., 2004). The fact that reward-related activity of VTA neurons in the current study was sensitive to changes in visuo-spatial and reward components of the context provides further compelling evidence that contextual information plays a significant role in the reward-coding properties of VTA neurons. Interestingly, the most dramatic changes in reward-related responses only occurred following context manipulations that tested subjects' spatial memory ability (i.e., Darkness and Reward Location Switch conditions). These two context manipulations, however, induced opposite behavioral effects (i.e., spatial working memory was degraded in dark testing conditions, but enhanced when reward locations were changed) while inducing qualitatively similar changes in unit activity. Consistent with one recent report (Roesch et al., 2007), the activity of VTA neurons may not directly reflect the behavioral decisions made during performance of a spatial working memory task. Therefore, it is important for future experiments to directly test whether aspects of VTA neuronal activity underlie spatial working memory ability, or whether this function is governed by target structures, such as NAc or prefrontal cortex (Floresco et al., 1999).

Lisman et. al. (Lisman and Grace, 2005) proposed that detection of an altered context results in increased dopamine release in hippocampus, manifested by a net increase in VTA dopamine cell firing. However, the context manipulations in the current study resulted in both increases and decreases in reward-related VTA activity. Assuming that at least some of these neurons were dopaminergic, this is perhaps not entirely surprising. If a new long-term memory needs to be formed, it would be advantageous to

have some neurons decrease activity, as this would allow for the long-term depotentiation (LTD) of specific hippocampal synapses (Sajikumar and Frey, 2004) that were components of the familiar context representation. This would likely aid in pattern separation processes and increase the specificity of the hippocampal representation of the contextual changes.

The fact that we found movement and reward correlates to be differentially affected by context changes indicates that the movement code does not serve as the sole predictive cue for reward. It is well known that hippocampus is a critical component of a larger neural system important for forming context-specific memories (Jeffery et al., 2004; Mizumori et al., 2007b). Although a functional connection between hippocampus and VTA was not directly tested in the current study, elimination of visuo-spatial cues (i.e., darkness) and alterations of reward locations result in changes in hippocampal place cell activity (Puryear et al., 2006; Smith and Mizumori, 2006a). Accordingly, the contextual manipulations employed in this study likely produced an altered pattern of hippocampal activity, which may have resulted in changes in the reward-related activity of VTA neurons.

The apparent context-independent movement coding of VTA neurons suggests that reward and movement information can be independently regulated during asymptotic spatial working memory performance. If these representations reflect learned behavioral responses, it is possible that the movement codes are context-dependent during new learning. Such representations may become context-independent to reflect the more automatic nature of behavioral control during asymptotic performance in this task. The prefrontal cortex is one possible source of movement information (Jackson and

Crossman, 1983; Carr and Sesack, 2000), since the activity of neurons in this area seem to be related to several aspects of an animals' movements under similar testing conditions (Jung et al., 1998; Pratt and Mizumori, 2001; Euston and McNaughton, 2006). It will therefore be important to test whether movement-related activity of prefrontal cortical neurons is sensitive to the same changes in context information.

Chapter 4: The Reticular Formation and Reward Prediction

The capacity of an organism to respond appropriately to environmental stimuli depends on the ability to detect changes in the outcome of its behavior. The mesocorticolimbic dopamine system is thought to be central to this function (Wise, 2004; Fields et al., 2007). Dopamine neurons in the ventral tegmental area (VTA) and substantia nigra *pars compacta* (SNc) increase activity relative to the presentation of cues that predict rewards and rewards of greater value than expected, and decrease activity relative to rewards of less value than predicted (Nakahara et al., 2004; Bayer and Glimcher, 2005; Pan et al., 2005; Tobler et al., 2005). This activity is thought to be involved in a computation about errors in the prediction of reward (Schultz and Dickinson, 2000) that can be used to correct behavior. A central issue relevant to the behavioral and computational interpretation of dopamine signals is whether prediction error signals are generated by dopamine neurons, *per se*, or by cells in 'upstream' brain areas.

Recent data suggest that brain areas afferent to dopamine neurons generate, or participate in, reward prediction error computations. Lateral habenula, which provides inhibitory inputs to VTA and SNc dopamine neurons (Herkenham and Nauta, 1979; Christoph et al., 1986), has recently been shown to be a potential source of reward prediction error signals (Matsumoto and Hikosaka, 2007). A similar finding has been demonstrated in the pedunculopontine tegmental nucleus (PPTg), which is an important regulator of dopamine neuron activity (Floresco et al., 2003). PPTg neural responses varied according to whether or not the animal received expected rewards (Kobayashi and Okada, 2007).

As part of a larger study investigating the role of VTA in context-dependent spatial working memory (Puryear et.al., submitted), we recorded the activity of neurons in the magnocellular region of the mesencephalic reticular formation, or MRNm (Swanson, 2003) which provides glutamatergic input to VTA (Geisler et al., 2007). The reticular formation is thought to be important for modulating arousal and vigilance levels necessary for attending to and acting upon salient stimuli (Pragay et al., 1978; Mesulam, 1981). Thus, MRNm is in a prime position to provide excitatory drive to VTA dopamine neurons when the outcome of behavior does not meet expectations, and therefore may be a source of reward prediction error signals. Accordingly, we investigated whether MRNm neurons exhibited reward related activity, and whether this activity was related to the ability to predict acquisition of reward.

METHODS

Subjects

Four male Long-Evans rats (4-6 months old from Simonson Labs, Gilroy, CA) were housed individually in Plexiglas cages in a temperature and humidity-controlled environment (12:12 hr light:dark). Food and water were provided *ad libitum* for 5 days prior to being handled daily and reduced to 85% of *ad libitum* feeding weights. Animal care and use was conducted according to University of Washington's Institutional Animal Care and Use Committee guidelines.

Differential Reward Spatial Working Memory Task

Rats were habituated to the testing environment and trained to perform a differential reward, win-shift spatial working memory task using radial maze procedures reported previously (Pratt and Mizumori, 2001; Puryear, submitted). Briefly, prior to the

start of each trial, the end of each of the eight maze arms was baited with either a large (5 drops) or small (1 drop) amount of chocolate milk on alternating arms. Maze arms containing large or small amounts of reward were counterbalanced across rats and held constant throughout training. Trials started with a Sample Phase by presenting four maze arms (two large and two small reward arms; individually and randomly selected) to the rat. Immediately after presentation of the fourth arm, a Test phase began by making all maze arms accessible so the rat could collect the remaining rewards. The trial ended once all arms were visited. The rat was then confined to the center of the maze for a 2 minute inter-trial interval. Arm re-entries were counted as errors. Once the rat exhibited consistent task performance, recording electrodes were surgically implanted.

Single-unit recording

Details concerning the construction of recording electrodes and microdrives and surgical procedures can be found in previous reports (McNaughton et al., 1983b; Puryear et al., 2006; Puryear, submitted). Briefly, rats were chronically implanted with either eight stereotrodes (four/hemisphere) or four tetrodes (two/hemisphere), centered around the following coordinates relative to bregma: -5.25 mm posterior, 0.7 mm lateral, 6 mm ventral (Swanson, 1998). One week of free feeding was allowed for rats to recover from surgery before recording experiments began.

Recordings were performed as described previously (Puryear et al., 2006; Puryear, submitted). If no clear spontaneous neural activity was encountered, electrodes were lowered in ~25 μm increments (up to 175 μm per day) until unambiguous, isolatable units were observed. Single units were isolated from multiunit records using standard cluster-cutting software (MClust; A.D. Redish, University of Wisconsin). A

template-matching algorithm (written by Chris Higginson) was also used to facilitate separation of unique spike waveforms. We only included cells with a high signal-to-noise ratio (>3:1), exhibited stable clusters throughout the recording session, and had clear refractory periods in the inter-spike interval histograms following cluster cutting.

Histology

The final position of each stereotrode was marked by passing a 25 μA current through each recording wire for 25 sec while rats were under 5% isofluorane anesthesia. Rats were then given an overdose of sodium pentobarbital and transcardially perfused (0.9% buffered saline, followed by 10% formalin). Electrodes were retracted and the brain was removed and allowed to sink in 30% sucrose-formalin. Coronal sections (40 μm) were sliced with a cryostat and stained with cresyl violet. Recording locations were verified by comparing depth measurements and reconstructions of the electrode tracts. Only cells determined to be located in MRNm (Swanson, 2003) were considered for analysis.

RESULTS

During asymptotic performance, rats performed five trials of the spatial working memory task (Baseline trials), committing 0.86 ± 0.2 (mean ± sem) errors per trial. Importantly, rats demonstrated the ability to discriminate large and small reward locations. There was a significant negative correlation between the first four Test Phase arm choices (i.e., first, second, third, and fourth arm choice) and the probability that the arm chosen contained a large reward (Spearman's $\rho = -0.65$, $p < 0.001$), indicating that rats reliably visited large reward arms before small reward arms during the Test Phase of each trial.

A total of 18 cells localized to MRNm were recorded while rats performed the task. Of these, one cell was omitted from analysis due to a very low average firing rate (~0.2 spikes/sec), yielding 17 cells included in the following analyses. Figure 3.1a depicts the distribution of cells localized to MRNm. These cells exhibited a range of average firing rates, spike durations (defined as the time from the start to the end of the action potential, Figure 3.1b), and firing patterns (see Figure 3.1c for representative inter-spike interval and autocorrelation histograms).

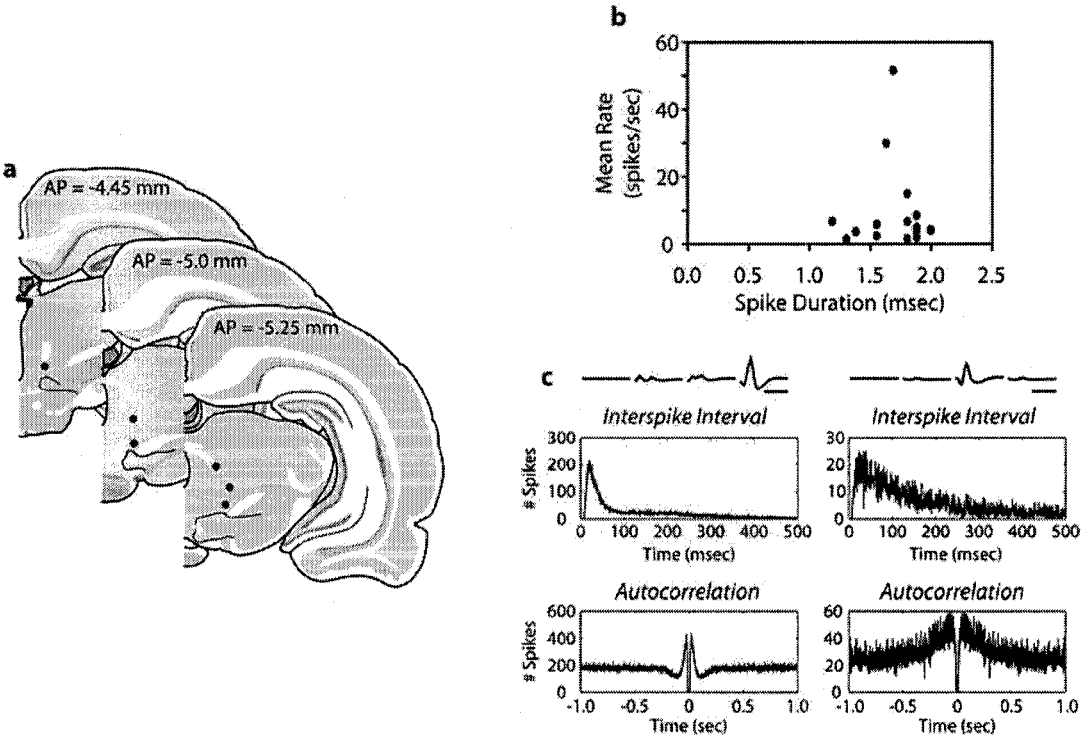

Figure 3.1:
Histology and basic firing properties of MRNm neurons. A) Distribution of cells localized to MRNm. Each dot may represent the location of more than one neuron. Coronal slices adapted from Swanson, 2003. B) Distribution of average firing rates and spike duration of MRNm neurons. Most cells fired less than 10 spikes/s and exhibited waveform durations between 1.5 and 2.0 msec. C) Examples of two MRNm neurons. Top row shows their average waveform on each wire of the tetrode, scale bar = 1 msec; middle and bottom rows depicts their inter-spike interval and autocorrelation histograms, respectively.

Reward-related neural activity was obtained by placing rewards in small metal cups mounted to the end of each maze arm and connected to the recording equipment (custom designed by Neuralynx, Inc.), which served as 'lick-detectors'. An event marker was automatically inserted into the data stream when the rat licked the cup, providing an instantaneous measurement of the time the rat first obtained reward.

In order to determine whether MRNm neurons exhibited significant reward-related activity, peri-event time histograms (PETHs) were constructed (50 msec bins, ± 2.5 s around each reward event). A cell was considered to have a significant excitatory reward response if it passed the following two criteria: 1) the cell had a peak firing rate within ± 150 ms of reward acquisition and 2) the peak rate was >150% of it's average firing rate for the block of trials. These criteria were applied to PETHs collapsed across reward amounts, and separately for large and small reward events. Overall, 47% (8/17) of MRNm neurons were found to exhibit significant excitatory responses upon acquisition of reward (Figure 3.2d). Of these cells, most (88%, 7/8) were found to fire relative to acquisition of only large rewards (for example, Figure 3.2a-c), while the remaining neuron fired relative to acquisition of both reward amounts. No cells were found to fire preferentially to acquisition of only small rewards. Aspects of animals' movement (e.g. velocity) were not found to be a major contributor to the firing patterns of MRNm neurons during performance of the spatial working memory task (data not shown). Therefore, it appears that MRNm unit activity is predominantly biased to represent higher reward values.

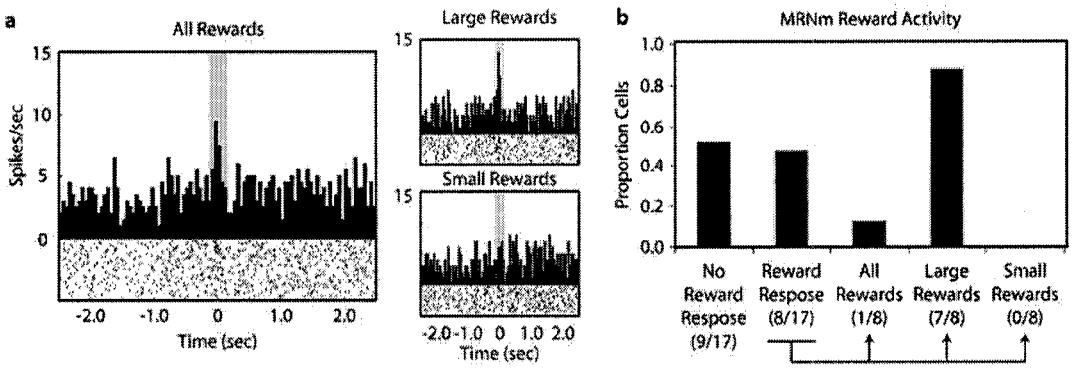

Figure 3.2:
Baseline reward-related activity of MRNm neurons. A) Peri-event time histograms of one cell that exhibited a short-latency, excitatory response upon acquisition of rewards (t_0, bin width = 50 ms). Left histogram shows only a modest excitatory response when considering all rewards together. However, top and bottom right histograms show that the reward-related firing occurred upon acquisition of large and not small rewards. Grey shaded areas indicate time periods analyzed for significant increases in firing rate. B) Population summary of the proportion of MRNm neurons that demonstrated significant reward-related activity.

In order to determine whether MRNm reward-related activity was associated with reward prediction, we tested unit responses to unexpected alterations of reward outcome or elimination of visuo-spatial information important for reward prediction. To do this, we allowed the rat to perform a second block of 5 trials with either the locations of large and small rewards switched (Reward Location Switch condition), with two rewards (one large and one small, randomly selected) omitted from the Study Phase of each trial (Reward Omission condition), or with the maze room lights extinguished (Darkness condition). Importantly, each of these manipulations created situations in which reward prediction errors likely occurred. Overall, these three testing conditions created the following situations, respectively: a mis-match between the locations of large and small rewards, a decreased probability of obtaining a reward, and a situation in which rats are not able to discriminate between arms associated with large and small amounts of reward

(Puryear et al., submitted). Therefore, positive prediction errors could occur when the animal received a large amount of reward on an arm previously associated with a small amount, when the rat retrieved rewards after visiting arms in which reward had been omitted, and when the rat obtained a large reward in darkness. Negative reward prediction errors could occur when the animal received a small amount of reward on a maze arm previously associated with a large amount, when the rat visited an arm that did not contain a reward, and when the rat obtained a small reward in darkness.

Eight cells with significant responses to large rewards were recorded during these tests (2 during Reward Location Switch, 4 during Reward Omission conditions, and 2 during Darkness conditions). In order to determine whether the reward manipulations affected the reward-related activity of these cells, a Reward Activity value (*RA*) was first calculated, which was the average firing rate in the ± 150 ms around the time of acquisition of rewards, expressed as a percent change relative to the cell's average firing rate for each block of trials. These values were normalized to the maximum *RA* value observed, yielding a normalized *RA* value for the first and second block of trials (RA_{n1} and RA_{n2}, respectively). These calculations were made for large and small rewards separately, and for non-rewarded arms in the Reward Omission condition.

We then created scatter plots of RA_n's for each block of trials. If reward-related activity was independent of the expectation of the reward received, RA_{n1} and RA_{n2} should be similar in each block of trials. As can been seen in Figure 3.3a, the reward-related activity was consistently higher when rats received more reward than expected in the second block of trials. Conversely, Figure 3.3b clearly shows that neural activity was consistently suppressed when rats received less reward than expected. These differences

in reward-related firing were quantified by calculating the distance of each data point to the diagonal (i.e., the Reward Activity Change Index, or RACI):

$$RACI = \frac{\sqrt{2(RA_{n1} - RA_{n2})^2}}{2}$$

Directionality of the change in reward activity was taken into account in order to discern between increases and decreases in firing rate. A one-sample T-test ($\alpha = 0.05$) indicated that average RACI values were significantly increased when rats received more reward than expected ($t_7 = 2.73$, $p < 0.03$), and significantly decreased when rats received less reward than expected ($t_7 = -4.88$, $p < 0.001$). These results are consistent with positive and negative reward prediction error signals, respectively. An example of an MRNm neuron that exhibited both positive and negative prediction error-related activity in the Reward Location Switch condition is depicted in Figure 3.3d.

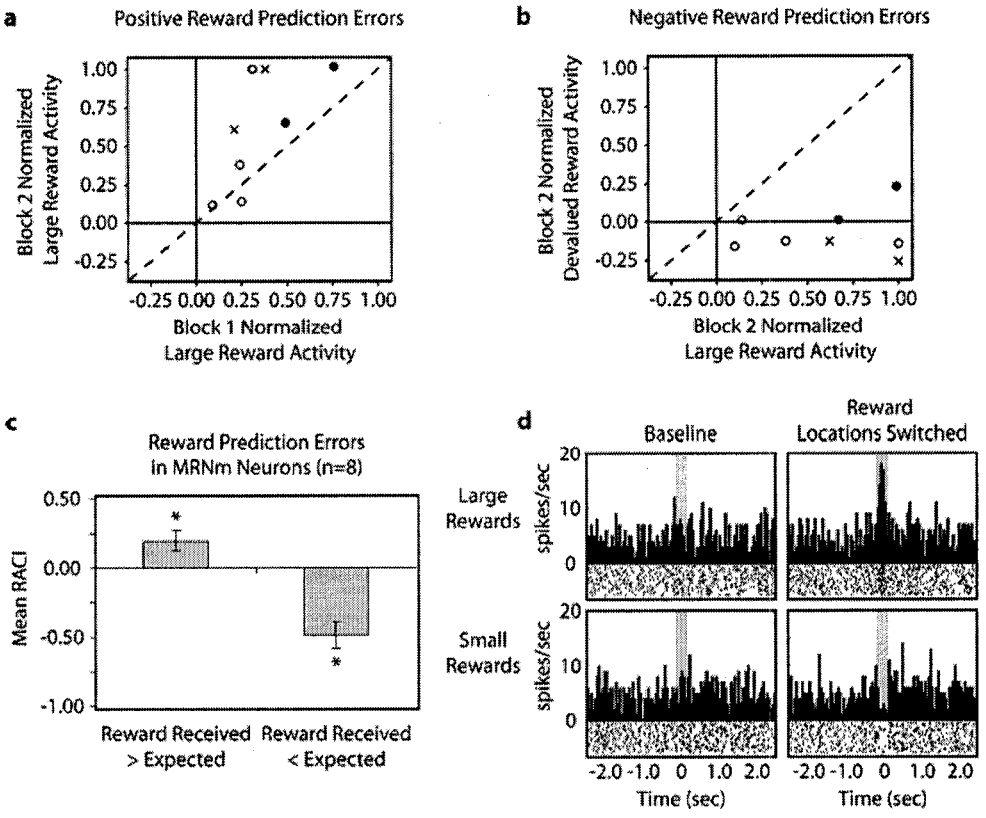

Figure 3.3:
Reward prediction errors in MRNm neurons. A) Plotted is each neuron's normalized large reward activity (RA_N, defined in Methods) for each block of trials. The reward activity during Block 2 (y-axis) represents activity at times when more reward than expected was obtained. Note that reward-related activity during these times is consistently more robust than during times in which the rat received the expected reward (Block 1), indicating that positive reward prediction errors occurred. B) Plotted are RA_N values for rewarded and devalued arms in Block 2 (x and y axes, respectively). Devalued arms include arms associated with a large amount of reward, but baited with a small amount of reward, arms in which reward was omitted, and arms containing small rewards visited in darkness. Note that reward-related activity on devalued arms is consistently suppressed, indicating that negative reward prediction errors occurred. C) Average changes in reward activity (RACI, defined in Methods) for times in which the rat obtained more and less reward than expected. Asterisks indicate significant differences ($p < 0.05$), error bars indicate s.e.m. D) An example of one neuron that did not respond to acquisition of rewards during the first block of trials. When the locations of large and small rewards were switched, however, the cell developed an excitatory response to acquisition of large rewards on arms previously associated with small amounts of reward (positive reward prediction error). Furthermore, the firing of the cell was inhibited upon acquisition of small rewards on arms previously associated with large amounts of reward (negative reward prediction error).

DISCUSSION

We demonstrate here that a large proportion of MRNm neurons may be involved in computations about reward acquisition. Similar to dopamine neurons (Tobler et al., 2005), the majority of reward-related MRNm neurons preferentially fired relative to acquisition of large amounts of reward. To our knowledge, this is the first demonstration of discriminative reward responses of reticular formation neurons, and it highlights a novel role for the reticular formation in reward value representations. Furthermore, these data suggest that MRNm neurons represent reward prediction error signals similar to those seen in dopamine neurons (Nakahara et al., 2004; Bayer and Glimcher, 2005; Pan et al., 2005; Tobler et al., 2005). It is important to note that in this initial sample, there was remarkable overall consistency and reliability of the positive and negative reward prediction error signals by MRNm neurons. This is similar to the homogeneity of dopamine neuron responses, suggesting that reward prediction may be a major function of the overall population of MRNm reward-related neurons. Nevertheless, further parametric studies are necessary to determine whether MRNm neural activity conforms to the same basic firing profiles that have been well described for dopamine neurons (i.e., predictive cues and reward probabilities).

The reticular formation has traditionally been thought to be important for initiating general arousal states. This is in part due to initial reports of changes in unit activity during transitions from sleep to wakefulness (Huttenlocher, 1961; Kasamatsu, 1970; Manohar et al., 1972). In addition, a more specific role for the reticular formation in attention has been described in primates performing visual discrimination tasks (Pragay et al., 1978; Fabre et al., 1983). This is consistent with reports of sensory neglect

following reticular formation lesions (Watson et al., 1974). Together, these foundational data suggest that reticular formation may function to enhance the overall level of arousal and vigilance necessary for attending to and acting upon salient stimuli (Mesulam, 1981). Accordingly, changes in reward-related MRNm neuronal activity could provide an important signal indicating that the contingencies of recently executed behaviors have changed.

The striking similarity of the reward prediction error signals of MRNm neurons reported here suggests that MRNm, along with brain regions such as lateral habenula (Matsumoto and Hikosaka, 2007) and PPTg (Kobayashi and Okada, 2007) may contribute to the generation of reward prediction error signals. Furthermore, these data suggest the possibility that such signals are a general property of a large network of midbrain structures. Given that the projections from the reticular formation to VTA are glutamatergic (Geisler et al., 2007), it is possible that the changes in reward-related activity of MRNm neurons, in concert with PPTg, could provide an excitatory component of the reward prediction error signal. In combination with inhibitory inputs from lateral habenula, this may then selectively activate dopamine neurons to initiate the coordinated selection of appropriate behaviors in response to changes reward outcome (Humphries et al., 2007).

Chapter 5: Conclusions

As we and others have proposed, hippocampus, as part of a larger system important for episodic memory, makes computations important for determining whether an expected context has changed (Lorincz and Buzsaki, 2000; Vinogradova, 2001; Lisman and Grace, 2005; Smith and Mizumori, 2006b; Mizumori et al., 2007b, a). The studies presented in this dissertation provide some insights into potential neural mechanisms involved in this process. The alterations of context information utilized throughout these studies were chosen because each of them induced a situation in which the animal was likely less able to predict when and/or where it would receive reward. These studies provide compelling evidence for specific properties of hippocampal place fields that are important for context-dependent spatial memory, that VTA neural activity is, indeed, gated by contextual information, and that MRNm may have a novel and important role in generating prediction error-related signals. The nature of the manipulations, however, likely engaged hippocampal-midbrain circuitry in different ways. When visuo-spatial information was eliminated in darkness, it is possible that hippocampus initially 'noticed' the change in context, as proposed by Lisman et al. (2005). However, since alterations of rewards could only have been detected by the animal upon encountering a different reward than expected, it is possible that these manipulations initially engaged VTA and/or MRNm neuronal activity, thereby causing a new context representation to be formed.

Alterations of visuo-spatial information

In dark testing conditions, hippocampal afferent information from sensory and association areas of cortex would be changed due to the lack of visuo-spatial information. This altered input to hippocampus would then produce a different spatial reference framework, perhaps relying more on non-visual information (i.e., proprioceptive, tactile, and/or olfactory information) that the animal would attempt to use to guide its behavior appropriately during dark trials. This altered output of hippocampus could then have the net effect of changing the firing patterns (i.e., burst firing) of VTA and/or MRNm neurons. It is important to note that the changes in activity seen in these midbrain areas, potentially signaling prediction errors, are not evident until the animal obtains a poorly predicted reward. Thus, it appears that the alterations in reward responses in dark testing conditions were not caused by the loss of visuo-spatial information, *per se*. Rather, they may have been due to changes in afferent information from hippocampus regarding the expected outcome of the animal's behavior that normally would have gated the reward related activity of VTA and MRNm neurons. Since the change in visuo-spatial information produced an altered representation of the spatial context in hippocampus, different populations of place cells would be active as the animal approached the reward. Therefore, the altered hippocampal output would have necessarily changed the gating function, thereby allowing VTA and/or MRNm neurons to exhibit prediction error signals when the animal obtained rewards. This could then have initiated the process of selecting new behaviors/strategies that would maximize the likelihood of obtaining reward via NAc and globus pallidus (GP, the main output area of basal ganglia) (Grace et al., 2007).

According to Lisman and Grace (2005), altered dopamine release from VTA would enable plasticity mechanisms in hippocampus to help sculpt and solidify the altered representation generated when the lights were extinguished. As mentioned above, the altered hippocampal context representation would be based on qualitatively different, non-visual sources of information. If this strategy is successful, characteristics of hippocampal place fields (i.e, their locations and/or reliability) would be altered, but as long as the new representation is specific enough, the animal can adaptively use these sources of information to support the short-term memory functions necessary to perform the task accurately (Puryear et al., 2006). Although an intriguing possibility is that the generation of a new and specific spatial context representation requires a net increase in dopamine release in hippocampus (Lisman and Grace, 2005), it appears that reward-related VTA neurons respond heterogeneously (i.e., increases and decreases) in dark testing conditions. Assuming that at least some of the VTA neurons were dopaminergic, this finding is perhaps not entirely surprising, since a new representation necessitates that some synapses become potentiated and depotentiated (Sajikumar and Frey, 2004), thereby facilitating a pattern separation process (Tsodyks, 2005; Leutgeb et al., 2007) that allows a more unique context representation to be formed. Alternatively, it is possible that only the VTA neurons that increased reward-related activity in dark testing conditions were dopaminergic, whereas neurons that did not were either glutamatergic or GABAergic. Since very little is currently understood about these hippocampal afferents from VTA, it is critical to further explore the potential roles these cell types play in hippocampal function.

Unexpected alterations of reward

The manipulations of expected rewards employed in these studies provide an alternate view on how hippocampal-midbrain circuitry can be engaged when context information is unexpectedly changed. This is due to the fact that the animal is only able to detect that there has been a change in the context once it does not receive the reward it expected. Therefore, it is likely that the change was first detected by reward-related areas, such as VTA and/or MRNm. Given that VTA receives glutamatergic projections from MRNm (Geisler et al., 2007), it is possible that the changes in reward-related activity in VTA neurons were driven by MRNm reward prediction error-related activity. It is likely, however, that this excitatory drive produced changes in VTA neuronal reward responses in conjunction with prediction errors from lHb (Matsumoto and Hikosaka, 2007). Although there may be evidence for an involvement of PPTg in signaling prediction errors, the pattern of activity seen during times the subject does not receive expected reward is more protracted in time (Kobayashi and Okada, 2007), as opposed to the short latency (~150-200 msec) responses seen in MRNm and lHb. Therefore, it is possible that PPTg may function more to regulate the amount of burst activity in VTA neurons in response to afferent information (Floresco et al., 2003). The resulting changes in dopamine release would then have led to LTP/LTD in specific hippocampal synapses that may facilitate the formation of a new context representation. With repeated exposure to the new reward contingencies, the formation of a new stable and specific context representation may then be facilitated by a more tonic level of dopamine release (Smith and Mizumori, 2006a). In parallel with the changes in hippocampal activity, phasic dopamine release in NAc is thought to shift processing in favor of hippocampal afferents

(Grace et al., 2007). This could effectively 'tune' the selection of new behaviors and/or strategies towards the newly developing context representation. It is interesting to note that when the locations of large and small rewards were switched, rats were not able to adjust their behavior accordingly. This indicates that the plasticity mechanisms involved in selecting and consolidating new appropriate behaviors takes longer than the allotted five trials. Presumably, with more exposure to the new reward locations, rats would eventually adjust their behavior accordingly. Therefore, it is important to determine whether the development of the behavioral adaptation is accompanied by a corresponding change in VTA and MRNm neuronal activity.

Although the studies provided in this dissertation highlight some novel aspects of hippocampal-midbrain circuitry that may be involved in context-dependent spatial memory, they also highlight several 'holes' in our current understanding of the circuitry involved in these functions. First, and foremost, the precise role MRNm plays in spatial memory is untested. Although unilateral lesions of MRNm can produce hemi-spatial neglect (Watson et al., 1974), it is difficult to determine how the reward-related neuronal activity of MRNm neurons observed here relates to the general 'activational' functions the reticular formation is thought to be involved in (Pragay et al., 1978; Mesulam, 1981). As such, it is important to determine whether loss of MRNm function impairs spatial learning and/or spatial memory abilities under conditions of contextual change. Studies such as these will not only extend our current understanding of the role reticular formation plays in hippocampal-dependent mnemonic functions, but also the potential role MRNm plays in driving VTA neuronal activity. Given that MRNm is strategically positioned to drive prediction error-related activity in VTA, it is important to determine

whether the activity of MRNm neurons is also modulated by reward probability and reward value, as has been demonstrated in putative dopamine neurons. Studies such as these will also further our understanding of the neural basis prediction error signals that presumably underlie adaptive behaviors in changing and uncertain environments.

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Curriculum Vitae

Education:

University of Washington, Seattle, WA
Graduate Program in Psychology (Behavioral Neuroscience), September 2002-June 2008
Advisor: Dr. Sheri J. Y. Mizumori
Date Ph.C. received: December 2005
Ph.D. received: June 2008

University of Vermont, Burlington, VT
Bachelor of Science in Psychology, Minor in Chemistry, May 2000
Co-advisors, Dr. Bruce S. Kapp and Dr. Mark E. Bouton
Honors: Dean's List - fall 1998, National Honors Society for Psychology-inducted 1998

Research Experience:

Doctoral Dissertation Research, Department of Psychology, University of Washington, September 2002-June 2008
- Dissertation Title: A Role for Hippocampal and Midbrain Neural Processing in Context-Dependent Spatial Memory

Research Technician I, Department of Psychology, University of Washington, June 2001-September 2002
- Performed behavioral and electrophysiological studies on the contribution of the hippocampus to spatial learning and memory
- Performed general laboratory duties (animal care, ordering supplies)

Laboratory Technician II, under Dr. George Wellman, Totman Lab for Cerebrovascular Research, Department of Pharmacology, University of Vermont, May 2000-June 2001
- Performed functional studies on small diameter (100-200 μm), pressurized rat and rabbit cerebral, and human oumental and cerebral arteries, along with data analysis of results
- Assisted during surgery for rabbit model of subarachnoid hemorrhage, performed surgery for angiography
- Perform general laboratory duties (ordering supplies, chemicals)

Work-Study Lab Assistant, Research Lab Assistant under Dr. Bruce S. Kapp, Professor of Psychology, University of Vermont, 1997-2000.
- Worked with Dr. Mary Cain investigating the contribution of the amygdaloid central nucleus to conditioned thalamic arousal.
- Performed single cell recording in the lateral geniculate nucleus during stimulation of the central nucleus of the amygdala and fear-induced arousal in rabbits.

Praecis Pharmaceutical Inc., Boston MA, Internship May-September 1999
- Worked on drug development team investigating novel compounds to treat Alzheimer's disease

- Performed brain uptake studies on rats and mice, surgery for brain perfusion, necropsies, capillary depletion, blood collection and analysis, and data analysis.

Teaching Experience:
Guest Lecturer on Learning and Memory, Biopsychology (Fall 2007)
Teaching Assistant, Biopsychology (Spring 2003, 2005-2007, Fall 2007, Winter 2008)
Teaching Assistant, Cognitive Psychology (Winter 2005)
Teaching Assistant, Abnormal Psychology (Winter 2004)
Teaching Assistant, Introductory Psychology (Fall 2003)

Awards:
Graduate Student Award for Excellence and Innovation (Fall 2007)

Publications:
Book Chapters

Mizumori, S.J.Y., Smith, D.M, **Puryear, C.B.** (2007) *Mnemonic contribution of hippocampal place cells.* In: Neurobiology of learning and memory, second edition. Eds: Kesner, R.P. and Martinez, J.L. Academic Press.

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