Single Unit Recordings of Rat Lateral Habenula during a Navigation-based Spatial Memory Task

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Abstract

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Psychology

In recent years, the lateral habenula (LHb) has become an area of great interest, as in vivo electrophysiological studies in head-fixed primates revealed the presence of neurons that respond differentially to rewards, punishment, and their cues-- in a manner opposite to the well-characterized dopaminergic neurons in the ventral tegmental area (VTA). Furthermore, these responses encode reward/punishment magnitude and are dependent on the outcomes of previous trials. Thus the LHb may be a generator of error prediction signals (Hikosaka, 2010). The lateral habenula is a point of convergence for basal ganglia and limbic circuits, which then projects to midbrain neuromodulatory systems. One functional connection of great interest includes inhibitory connections between LHb and VTA. LHb may also be part of action selection neural circuitry that is guided by motivation. We studied the role of the LHb in motivated behaviors in a semi naturalistic form. In particular, we conducted single unit recordings in LHb as Long Evans rats

performed a navigation-based spatial memory task on a radial arm maze. Analyses of neural data confirm the existence of RPE encoding cells; however, the majority of LHb neurons recorded contain movement, particularly velocity, related correlates. Although the habenula has been found to be involved in many behaviors, Hikosaka has also proposed that the primary function of the lateral habenula is to suppress motor function under unfavorable conditions. The movement related cells found here may be involved in monitoring overall activity levels or encode specific aspects of behavior for action-specific learning.

Introduction

The habenula (Hb), a highly conserved structure, is part of the dorsal diencephalic conduction system (DDCs), which connects limbic forebrain structures to major neuromodulatory systems in the limbic midbrain. Conveniently (or perhaps not), the habenula receives and transmits information through two main fiber bundles. It receives projections from the limbic forebrain through the stria medullaris (SM), and relays information via the faciculus retroflexus (FR). The DDCs runs in parallel with the medial forebrain bundle (MFB), which gained much attention with the discovery of "pleasure centers" by Olds and Milner in the 1950s and became the primary target of studies regarding communication between the limbic forebrain and midbrain. Despite this, throughout the 20th century it became clear that the habenula had great effects on a variety of behaviors, whether through direct manipulation of the habenula itself, or through its more conspicuous afferent and efferent paths. The affected behaviors were broad, diverse, and not without conflicting accounts. They included olfactory guided behavior, mating, ingestion, maternal behaviors, endocrine control, attention, and behavioral responses to rewarding and aversive stimuli (Sutherland, 1982). Such varied accounts could be in part due to the fact that the habenula is comprised of two distinct structures, aptly named medial habenula (MHb) and lateral habenula (LHb). They have distinct cell types, cytoarchitecture, and mainly non-overlapping projections to major neuromodulatory systems each with their own widespread effects. Considering this, it is clear how pharmaceutical manipulation of "the habenula" or stimulation of the SM and FR would yield such diverse and elusive results.

The present study focuses on the lateral habenula (LHb). Broadly speaking, the LHb is a point of converging information from the basal ganglia and limbic system and projects indirectly to dopaminergic and sertonergic systems. More specifically, the LHb receives a large projection from the basal ganglia by way of the globus pallidus internal segment (GPi), or the rodent homologue, the entopeduncular nucleus. In rats, this projection is so strong, that nearly every entopeduncular neuron projects to LHb topographically (Sutherland, 1982). LHb also receives input from the diagonal band of Broca, lateral preoptic area, and lateral hypothalamus. LHb projects to the rostromedial tegmental nucleus (RMTg), which sends inhibitory connections to dopamine cells in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) and to serotonergic cells in the dorsal and medial raphe. The VTA and raphe also send connections back to the LHb, creating a possible feedback loop (Geisler & Trimble, 2008). The connectivity of this circuit implies that behavioral information from the basal ganglia and motivation from the limbic system meet in the LHb, which sends signals to widespread neuromodulatory systems, which are equipped to send feedback with information regarding the outcome of the behavior.

Since 2007, the lateral habenula (LHb) has received much attention for its possible role in processing reward information. This focus is due to results from primate and human imaging studies indicating that the LHb participates in generating reward prediction errors (RPEs)--a signal that indicates that a behavioral outcome is incongruous with the subject's expected outcome (Hikosaka, 2010). RPEs are essential for adaptive behaviors as they may be necessary for learning from mistakes. Reward predictions are the result of a computation with contributions from neural systems that process different

types of information (e.g. spatial, motivational, statistical, etc.). If a prediction is erroneous, then RPEs are a necessary signal to change the subsequent input strengths to those neural systems that generate future predictions (Glimcher et al., 2008). RPEs are canonically studied in midbrain dopaminergic neurons; however, evidence suggests LHb may be the source of the RPE signal observed in these neurons. The strongest evidence for the presence of RPEs in LHb come from *in vivo* electrophysiological recordings in head-fixed primates (Hikosaka, 2007), where LHb reward responsive neurons respond similarly, but in the opposite direction of VTA dopamine neurons.

The present study aims to confirm the presence of RPEs in the LHb of freely moving rats along with any other task related signals that may provide insight into the types of information processed by this structure. For this, a navigation based spatial working memory task that has been previously shown to reveal reward sensitive cells in various midbrain structures was used (Pratt & Mizumori, 2001; Puryear et al., 2010; Redila et al. 2015)

Materials and Methods

Subjects

Twelve male Long-Evans rats (350-500 g; Simonsen Laboratories) were individually housed in a temperature-controlled environment with a 12 h light/dark cycle. All experiments were conducted during the light phase. All subjects were given food and water *ad libitum* and handled for at least five days before behavioral testing began. During behavioral testing, rats were maintained at 85%-90% of their maximum free

feeding body weight. All animal care was conducted according to guidelines established by the University of Washington's Institute for Animal Care and Use Committee.

Differential-reward, spatial memory task

Behavioral training of a differential reward spatial memory task was conducted on an 8-arm radial maze as described previously (e.g. Puryear et al., 2010). The black Plexiglas maze consisted of a central platform (19.5 cm dia) that was elevated 79 cm off the ground with eight radially-extending arms (58 × 5.5 cm), see fig. 1. At the end of the maze arms was a small receptacle that contained, in an alternating fashion, either a small (.2 mL) or large (.6 mL) amounts of "reward" (50% diluted Ensure chocolate milk). Each maze arm was hinged such that access to the rewards were remotely controlled by moving the proximal segment up or down, connecting or disconnecting the ends of arms from the central platform. The maze was surrounded by black curtains with several visual cues for orientation.

Rats habituated to the radial arm maze through free exploration of the maze initially with randomly placed puddles of reward, then with rewards only at the end of arms. Once the animals consistently visited the ends of arms, training of the differential reward spatial memory task began. Each session consisted of two blocks of five trials. Each trial consisted of a study phase and a test phase. During the study phase of each trial, four of the eight arms (two large-reward and two small-reward arms) were pseudorandomly selected and presented individually. After presentation of the fourth arm, the test phase began by making all maze arms accessible at once. The rat was required to collect the remaining rewards. Revisits to previously visited end of arms

within a trial were coded as errors. When the animal returned to the central platform after visiting all eight arms, the arms were lowered so that the rat was confined to the platform, and the experimenter re-baited the arms. The locations of differentially rewarded arms were held constant in each rat throughout training but were counterbalanced across rats. Once rats made an average of one or fewer errors per trial on a training day, they underwent a surgical procedure for the implantation of recording electrodes.

During recording sessions, one of three manipulations were introduced for each session: reward switch, reward omission, or darkness. Block 1 consisted of baseline trials, where reward locations were kept identical to that during initial training. Experimental manipulations were conducted during block 2. Large and small reward locations were switched during "reward switch". In "reward omission" trials, two pseudorandomly chosen rewards (one large, one small) were omitted during the study phase. Reward switch and omission creates conditions where the animal would encounter larger than expected rewards, smaller than expected rewards, and unexpected no rewards. In the darkness condition, maze lights were turned off to eliminate visual cues.

Stereotaxic Surgery

Recording tetrodes were constructed from 20 μm lacquer-coated tungsten wires (California Fine Wire). Tetrodes were places in custom made drives and impedances were measured at 1 kHz then, if necessary, gold-plated or replaced such that final impedances were 0.2–1.2 MΩ. Rats were deeply anesthetized with isoflurane and an antibiotic (Baytril, 5 mg/kg) and analgesic (Ketoprofen, 1 mg/kg) were administered.

The skull was exposed and holes were stereotaxically drilled to allow for implantation of recording electrodes dorsal to the LHb (A-P: −3.5, M-L: ±.9, and D-V: 4–5 mm). Six animals were implanted with a 6-tetrode, linear bundle drive unilaterally, and six animals with two 2-tetrode microdrives bilaterally. A reference electrode was also implanted near the anterior cortex (ventral to the brain surface 1–2 mm), and a ground screw was secured to the skull. The drives were then fixed to the skull with screws and acrylic cement. Rats were allowed to recover for 5 days with free access to food and water.

After recovery, rats were returned to a food restricted diet and retrained until they completed 10 trials within an hour for two consecutive days. During retraining, tetrodes were slowly lowered to the LHb, no more than 320 μm/day. Once in the target region tetrodes were lowered in 40 μm increments in search of units, no more than 200 μm/day. Once a unit was found, recordings were conducted. At this point, experimental manipulations were also introduced in the behavioral task (see task description). Tetrodes were left in the same location for up to three sessions in attempt to record units across multiple experimental conditions.

Data Collection and Analysis

All cellular recordings were conducted using a Cheetah data acquisition system (Neuralynx). Signals were filtered between 0.6 and 6 kHz, and digitized at 32 kHz. Neuronal spikes were recorded for 2 ms after the voltage deflection exceeded a predetermined threshold at 500–7000× amplification. Animal position data was sampled at 30 Hz via a ceiling mounted video camera that tracked LEDs attached to a preamplifier on the animal's head. Signals were manually sorted using Offline Sorter (Plexon, Inc.)

that allows segregation of spikes based on clustering parameters such as spike amplitude, spike duration, and waveform principle components. Cells were further analyzed if the waveform amplitude was at least 1.5 times that of the background cellular activity, and if the cluster boundaries were consistent across the session.

The behavioral correlates of unit activity were analyzed using custom Matlab software (MathWorks Inc. Natick, MA). Position data was used to manually place event flags to mark various aspects of behavior throughout the task such as reward encounter, animal turns, inbound movement, errors, etc. Given our hypothesis that the LHb regulates VTA dopamine cell responses to reward, reward-related responding in LHb cells were evaluated using similar methods that were used to identify VTA reward responses in prior studies (e.g. Jo et al., 2013 and Puryear et al., 2010). In short, neural data were organized into perievent histograms (PETHs) that were centered around the time of reward encounters (±2.5 s; 50 ms bins). Cells were considered to be reward related if peak (or valley) firing occurred within ±150 ms of reward encounters, and the mean firing rate of the ±150 ms window around the reward encounter was over 150% or under 75% of the mean session firing rate.

Throughout the course of the experiment, it became clear that the LHb contained velocity correlated cells. Thus, firing rates of LHb neurons were correlated with the velocity of the animals as they traversed the maze. Based on animal tracking data, 'instantaneous' velocity of the animal was determined by dividing the distance between two points by the inverse of the video sampling rate (Gill and Mizumori, 2006, Puryear et al., 2010 and Yeshenko et al., 2004). Each cell's firing rate was then correlated with these velocity measures (Pearson's linear correlation; $\alpha = .05$) within the range of 1–30 cm/s.

Velocity analysis did not include times when the animal was not moving, for example during reward consumption.

Histology

After the completion of all recording sessions, tetrode locations were verified with marking lesions. Rats were deeply anesthetized with 4% isoflurane, and each tetrode was marked by passing a 15 μA current through each tetrode wire for 15 s. The animals were then given an overdose of sodium pentobarbital and transcardially perfused with 0.9% saline and a 10% formaldehyde solution. Brains were stored in a 30% sucrose in 10% formalin solution at 4°C for one week. The brains were frozen, and then cut in coronal sections (45 μm) on a freezing microtome. The sections were mounted on gelatin-coated slides, stained with cresyl violet, and examined under light microscopy. The locations of recorded cells were determined using standard histological reconstruction methods. Only cells verified to be recorded in LHb were included in the data analysis.

Results

Histology

Of the twelve animals implanted, lateral habenula placed tetrodes were confirmed in six of these animals, fig 2. In the LHb, a total of 36 unique units were recorded throughout this task. Many cells were recorded for multiple sessions (up to three) in attempt to capture their responses under various experimental manipulations. Units were considered the same cell if they were recorded at the same depth and had comparable waveforms. There was minimal ambiguity in this selection process with signals in the

lateral habenula being relatively sparse and often only one cell being recorded per session.

Firing Rates

Sample traces are shown in Figure 3. Mean firing rates ranged from .5 to 107.6 spikes/s. However, over half the sessions contained units with an average firing rate of less than 10 spikes/s. Figure 4 shows the distribution of mean firing rates for LHb cells throughout the course of the study. The wide range of average firing rates suggest that there were multiple cell types recorded throughout this study. Lower firing rates, which include the majority of the units recorded, are consistent with what others have found in *in vivo* (Sharp et al., 2006) and *in vitro* (Weiss & Veh, 2011) recording studies.

Reward and consumption: single unit data

Out of the 36 recorded cells, a single neuron met criteria for a negative reward prediction error cell; shown in figure 5. The cell was significantly inhibited at the time of reward encounter, and was excited when rewards are omitted (see methods for criteria).

Another neuron was found to track both velocity and reward consumption, shown in figure 6. The cell was significantly correlated with velocity (Pearson's $r = .90$, $p < .001$). It also exhibits firing when the animal is not moving, but consuming reward, with differential duration according to reward size. Excitation was not observed during reward omission, and the cell would start firing only after the animal started to move.

Movement related responses

Overall, 66% of LHb cells (23/36) were found significantly correlated with animal running speed. Of these running speed cells half were (12/23) positive correlates and half were negative correlates (11/23). Examples of unit data are shown in figure 7. Figure 8 is a scatterplot showing the stability of these correlations between blocks. Units included in the plot were found to be significantly correlated with animal running speed for the session. Different colors/shapes indicate the experimental manipulation conducted during the session. There does not appear to be of experimental manipulation for these running speed cells—many of these cells were recorded for multiple sessions and comparable correlations were found across experimental manipulations.

LFP Results: Theta power is correlated with animal running speed

Local field potentials (LFP) data for sessions containing units in the LHb in the theta frequency band (4-8 Hz) were also analyzed. Spectograms of this frequency band revealed possible velocity correlates. Theta power (dB) was found to be significantly correlated with animal running speed for 36 out of 51 analyzed sessions. To examine the stability of these correlations, sessions were then grouped by tetrode location, such that a tetrode held at the same depth for multiple sessions would be considered a single "unit". Grouped in this way, 18 out of 22 "units" were found significantly correlated with animals running speed. Figure 9 shows the stability of these correlations across blocks.

Discussion

This study shows that a majority of neurons in the LHb are tracking movement, with often high correlations (>.9) with running speed. The population of movement

correlates is split, with half these cells being positive correlates and half negative. In addition, theta data reveal that running speed is also represented at the population level. Regarding reward related activity, only two out of 36 total unique units were found to be reward related—one being linked to consummatory behavior in addition to velocity, and the other exhibiting RPEs, as described by the Hikosaka group in 2007. Although it is exciting to confirm the presence of RPEs in LHb cells, this is a considerably smaller proportion of cells than expected, as the Hikosaka had found over 80% of primate LHb cell activity to be related to rewards (Matsumoto & Hikosaka, 2007). Considering the differences in animal and task, there are a number of reasons why this could be the case.

The original task used by Matsumoto and Hikosaka was much more Pavlovian in nature. While LHb neurons did show some excitation during unrewarded trials, these neurons showed much greater responses to the cues that predict reward omissions. They also showed high levels of responding during the first trial where a reward was omitted; however, once the animal knows whether or not it will be rewarded and the outcome is congruous with the expectiation, there is little change from baseline at the actual outcome. Given that the animals in the present study are highly trained, this may why we failed to observe more responses directly at the time reward.

In addition, the task used in primates require the subject to be head-fixed, which would simplify movement related neuronal activity. In the present study, if the rat LHb were tracking some sort of discrete cue, it is possible that movement related activity of the LHb cells would mask these signals. This is not particularly probable, as the task was designed without explicit cues. However, it may be the case that movement itself is the most reliable cue for when rewards will be received. In our task, the animal is very well

trained, and although navigation is goal directed, some aspects of the task are stereotypical. For example, all rewards are the same distance away from the center; therefore, once a choice is made, animal trajectory becomes perhaps the most reliable reward predictor. Movement correlates in rat LHb have been previously reported (Sharp et al., 2006) during a pellet-chasing task, which encourages the animals to run in semi random trajectories. They found that ~10% of recorded neurons to be significantly correlated with running speed as compared to our 66%. If LHb neurons are indeed tracking reward cues, animal movement may be overrepresented in this task.

To dissociate predictive movement from pure movement, future studies should include an open field component. If a proportion of the movement correlates found in the present study were actually reward predicting cues, then a subpopulation of these cells would not exhibit velocity correlates if recorded during general ambulation. To further investigate reward related responses, future studies should also consider using a task featuring explicit cues for rewards in order to observe LHb responses to reward predicting cues. Recording in an operant chamber would be ideal for this purpose. Although operant chambers are far removed from naturalistic behaviors, it would allow for tighter control over expectations, precise timestamps for reward receipts, and would restrict animal movement to minimize movement related activity.

Despite the possibility of movement as a predictor of rewards, it is clear that a proportion of LHb cells are heavily modulated by movement. Cells found in this study contain both positive and negative running speed correlates, which suggests that there may be subpopulations that code for different movement parameters. These cells could be informing targets in other structures and/or other LHb cells or of ongoing behavior. If

the primary function of the LHb is to suppress movement during unfavorable conditions, as Hikosaka (2010) proposes, it would be adaptive for movement suppression cells to be informed of ongoing behavior. This information would be necessary for action specific learning; to discourage actions that lead to negative outcomes. It could also be helpful in something more mundane, such as timing movement suppression, as it may be adaptive to suppress movement during particular phases of action; for example, during locomotion, when four limbs are on the ground as opposed to two.

The LHb is quickly becoming a region of interest for its relevance in multiple psychiatric disorders including addiction, depression (Lecca et al., 2014; Proulx et al., 2014), and to a lesser extent, aspects of bipolar disorder (Savitz et al, 2013), schizophrenia (Shepard et al., 2006), and Parkinson's Disease (Luo et al., 2015). This is primarily due to LHb connectivity between the limbic forebrain and dopaminergic and serotonergic systems, which are strongly associated with these disorders. It appears that there is an eagerness to start the manipulation of the LHb for clinical purposes. In 2010, a patient received chronic deep brain stimulation in the LHb for treatment resistant major depression, which resulted in full remission. Placebo effects were excluded because a bicycle accident disrupted DBS, unknown to the patient, and depression systems returned. Symptoms were once again alleviated after DBS was restored (Sartorius et al., 2010).

Regardless, a deeper understanding of the LHb is essential for more refined therapies. Ten subnuclei have been described in the LHb (Geisler et al., 2003), but their behavioral relevance has not been studied. Perhaps within the next decade, modern techniques such as optogenetics and DREADDs will result in a clearer picture of the

lateral habenula. Reliably finding RPE cells in rat LHb is critical to advancing our knowledge of this structure to better define and characterize relevant circuits to a host of meaningful behaviors.

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