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# CHANGES IN NUCLEUS ACCUMBENS CORE FIRING PATTERNS DURING REWARD-RELATED EVENTS

by

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**ABSTRACT OF THE DISSERTATION**

CHANGES IN NUCLEUS ACCUMBENS CORE FIRING PATTERNS

DURING REWARD-RELATED EVENTS

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Dissertation Director:

Mark O. West, Ph.D.

Learning is characterized by the ability to appropriately respond to external cues that predict rewarding or aversive outcomes. The nucleus accumbens is a key mediator of reward prediction and reward-related behaviors. However, it is not well understood how accumbens neurons acquire and maintain their responsiveness to these events. In the present study, 56 nucleus accumbens core neurons were electrophysiologically recorded over 10 sessions when the animals were trained in a conditioning paradigm. Changes in firing rates of core neurons were analyzed over training days during the following events: reward cue presentation, cued and non-cued approaches towards the reward, reward consumption and reward seeking. As a population, core neurons displayed an increase in firing rate in response to presentation of the reward predicting auditory cue. The latency of core activity in response to the auditory cue was significantly reduced after animals acquired the task. Increased firing rates were

also observed during approach behaviors following the onset of cue, but not during spontaneous approaches to the reward delivery port in the absence of the cue. In contrast, a general decrease in firing rate was observed during cued entry into the port when animals consumed the reward, but not during non-cued entry into the port when the reward was absent. Heterogeneous activities were discovered during all reward related events (i.e. individual neurons increased or decreased firing rate from baseline with various magnitudes). Further, strong stability across training sessions was observed during reward consumption but not during other reward-related events. That is, individual core neurons consistently responded to reward consumption across training sessions, exhibiting a general inhibition. However, individual core neurons did not consistently respond to other reward related events (e.g. cue presentation and cued approach) from session to session, indicating the general neural excitation observed during these events was mediated by different neurons from session to session.

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# 1. Introduction

## 1.1 Afferents and Efferents of Accumbens Core

The convergence of cortical, limbic and dopaminergic inputs in the nucleus accumbens (Nacc) places the Nacc in a unique position for integrating environmental stimuli, such as a reward-related cue or palatable food, with an animal's internal state (e.g. memory of encountering a specific stimulus with satiation state). Upon receiving this information, the Nacc participates in assessing the value of environmental stimuli. Through its primary efferent target, ventral pallidum, the Nacc has direct access the motor system. This allows the Nacc to contribute to selecting an appropriate behavioral response to a stimulus. Thus, given its afferent and efferent connections, the Nacc is believed to translate reward-related information into reward-directed actions (Balleine, 2005; Kalivas & Nakamura, 1999; Mogenson et al., 1980).

The major population of NAcc neurons are medium spiny neurons, representing over 90% in rodents and over 75% in primates (L. Heimer et al., 1997). However, Nacc is subdivided into two immunohistochemically distinct subregions, core and shell (L. Heimer et al., 1991; Jongen-Relo et al., 1994; Zahm & Brog, 1992). Nacc core and shell exhibit differential afferent and efferent connections. First, The core receives cortical inputs originating from the dorsal prelimbic, anterior agranular insular, anterior cingulate, and perirhinal cortices. Shell receives cortical inputs originating from the infralimbic, dorsal peduncular, and posterior piriform cortices. Regions innervating both core and shell, such as orbital, posterior agranular insular, and entorhinal cortices, hippocampus, and basal

amygdala, innervate the accumbens in a topographic manner, differentiating the core and shell (Brog et al., 1993). Core and shell project to the ventral pallidum, ventral tegmental area, and lateral hypothalamus, but with different topographies. The core innervates the dorsolateral ventral pallidum, substantia nigra, and the entopeduncular nucleus, whereas the shell innervates the subcommissural ventral pallidum, ventral tegmental area, lateral hypothalamus, and extended amygdala (L. Heimer et al., 1991). Given their differential anatomical connections, we and others have shown that the accumbens subregions play differential roles in reward-based behavior(Ambroggi et al., 2011; Carelli & Wondolowski, 2006; Fuchs et al., 2004; Ghitza et al., 2004; Ghitza et al., 2004; Mendoza et al., 2005).

**1.2 Role of Accumbens Core in Reward-Related Processes**

Neurochemical manipulations to the core and the shell suggest the two subregions are involved in different aspects of reward related processes. Co-activation of D1 and NMDA receptors in the Nacc core is necessary for the acquisition of operant responding reinforced by natural reward (Kelley et al., 1997; Smith-Roe & Kelley, 2000). In addition, the integrity of the core is necessary for the reinstatement of natural reward seeking behaviors in response to learned cues (Floresco et al., 2008). Similarly, Nacc core is involved in reward related actions toward abused drugs. Inactivation of core impairs cocaine seeking when the animals were trained under a second-order schedule, and was also shown to abolish cue induced reinstatement to cocaine (Fuchs et al., 2004; Ito et al., 2004). Taken together, core neurons substantially modulate reward-

related behaviors. On the other hand, shell neurons are more sensitive to the valence of reward. Shell lesions impair the ability to choose a reward with a greater value (Albertin et al., 2000; Corbit & Balleine, 2011).

### 1.2.1 Accumbens Core and Reward-related Cues

The ability to recognize and appropriately respond to cues that predict specific outcomes is critical for survival. On the other hand, over-sensitivity to cues may be detrimental. For example, addiction to drugs is characterized by a long-lasting risk of relapse, often triggered by cues related to abusing these drugs (Hyman & Malenka, 2001). Emerging experimental evidence has suggested that the processing of predictive cues is mediated by Nacc core and the brain regions closely connected to Nacc under both physiological and pathological conditions. The ventral tegmental area (VTA) dopamine neurons, which project to the accumbens, display increased activity upon the presentation of a reward related cue at the late stage of conditioning, when the animal has acquired the cue-reward relationship (Schultz, 2002). The increased dopamine neuron activity induces increased dopamine release in accumbens (M. F. Roitman et al., 2004b). Consistent with the dopaminergic activity, neurons in the accumbens acquire sensitivity to an auditory cue in a pavlovian paradigm (M. F. Roitman et al., 2005). Besides its role in the acquisition of a cue-reward relationship for natural reward, the Nacc core is also implicated in cued induced reinstatement of cocaine seeking. Cocaine associated-cue presentation selectively elicits increased dopamine release in accumbens core, and core inactivation by a GABA agonist blocks cue induced reinstatement (Ito et al., 2000). Similarly,

selectively bred rats that exhibit high propensity to self-administer addictive drugs show super-responsiveness to cues, super-sensitivity to dopamine, and have a higher proportion of D2 receptors and more spontaneous dopamine release in accumbens core (Flagel et al., 2010). In conclusion, the Nacc is critical in cue processing but few previous studies, especially studies involving natural reward, distinguish between core and shell. The current study aims to examine the activity of accumbens core during cue presentation throughout the learning process.

### 1.2.2 Accumbens Core and Goal-Directed Behavior

The recognition of reward-predictive cues engenders selective reward-directed approach behaviors. Early in cue-reward training, few cue-induced responses are evoked, indicating a weak cue-reward relationship. As a result of repeated exposure to the cue-reward pairing, the likelihood of cue-induced approaches increases and asymptotes, indicating acquisition of the cue-reward association. Similarly, the rates of extinction differ between early and late stages of training indicating that different representations of cue-reward associations occur at different stages of learning (Allan, 1963; Gottlieb & Prince, 2012). The quantitative and qualitative differences in cue induced responses at different stages of learning imply different underlying neural mechanisms. Experimental evidence has identified the accumbens core as a neuronal substrate that promotes cue induced behaviors when a cue-reward relationship is well established. Animals that received core lesions after acquiring a Pavlovian conditioning task showed a reduction in the ratio of cue induced approaches

versus spontaneous approaches. Retraining was not sufficient to rescue such impairment (Parkinson et al., 1999). These results indicate that core is crucial in supporting reward-directed behaviors. Later studies pinpointed dopamine as one of the key neurotransmitters that mediate core's control of cue-induced approach behaviors to abused drugs (Bassareo & Di Chiara, 1999; Choi et al., 2005; Fuchs et al., 2004) and natural rewards (Wakabayashi et al., 2004). Consistent with the dopamine blockade results, elevated dopamine levels are observed during cue induced lever pressing reinforced by natural rewards (M. F. Roitman et al., 2004a). Causally, electrically-evoked dopamine release is capable of triggering cocaine seeking (Phillips et al., 2003). Interestingly, such dopamine signaling in the core during cue induced behavior does not translate into a homogeneous neural activity. Extracellular recordings during cue induced instrumental responses and cue guided choice behaviors reveals both increased and decreased changes in firing from baseline, as well as neurons that do not change firing, among core neurons (Ambroggi et al., 2011; Jones et al., 2010; Samejima et al., 2005). This heterogeneous response pattern in accumbens core persists into ventral pallidum, distributing diverse signals onto pallidomesencephalic and pallidothalamic targets (Root et al., 2013).

As mentioned above, cue induced responses at different stages of learning appear to reflect different, shifting internal representation of the CS-US relationship. That is, it is possible that cue induced responses are mediated by different neural mechanisms at different stages of training. Dopaminergic manipulations in the core at different stages of learning lend support to this

hypothesis. D1 receptor antagonists block both pavlovian conditioned approaches and spontaneous approaches early in training (day 3), whereas only spontaneous approaches are blocked after extended training (i.e., conditioned approaches are left intact by day 10; Jon Horvitz, personal communication). The fact that the same pattern of results is observed with systemic D1 antagonism (Choi et al, 2005) strongly implicates core mechanisms in these behavioral effects. Unfortunately, it is not yet clear how the differential dopamine involvement translates into neuronal activity in the accumbens core.

In order to clarify core's role in mediating cue induced responses at different stages of training, the current study aims to characterize core neurons' firing patterns during cue induced approach across training sessions. Firing patterns of individual neurons and of the whole sample of core neurons will be analyzed and compared among different stages of training. This will help elucidate the role of accumbens core in promoting conditioned approach behavior.

### 1.2.3 Accumbens Core and Reward Consumption

The accumbens core is also involved in reward consumption. Core lesions have been shown to reduce both food intake and body weight (Mendoza et al., 2005). Dopaminergic concentrations are enhanced in accumbens core during food consumption (Bassareo & Di Chiara, 1999). Both increases and decreases in neuronal activity during reward consumption are observed among neurons in accumbens core (Taha & Fields, 2005). The subpopulation of accumbal neurons that decrease firing rates were shown to be crucial to initiate and maintain feeding (Krause et al., 2010). Moreover, Pavlovian conditioning alters the

afferents to the core. Ventral tegmental area neurons display a dynamic change of activity over the course of conditioning. Reward delivery triggers an increase in dopamine neuron activity initially. Reward-related firing patterns decrease and cue-related firing patterns increase as the association between the cue and reward strengthens (Schultz, 2002; Stuber et al., 2008). Therefore, core neuron firing patterns during consumption may be influenced by conditioning by virtue of the altered dopaminergic input.

### 1.3 Heterogeneity of Accumbal Neural Activity

Task-responsive core neurons are highly heterogeneous, exhibiting any combination of firing patterns during different behavioral events as well as different directional changes in firing (e.g., increase from baseline, decrease from baseline, or no change) (Carelli & Wondolowski, 2006; Ghitza et al., 2004; Jones et al., 2010; Nicola et al., 2004). The heterogeneity in neuronal responses suggests that examining the activity of accumbal neurons in isolation might not reveal the holistic nature of accumbal processing. Therefore, the current study explored the neuronal activity of the whole sample of accumbens core neurons, in addition to individual neuron activity through different stages of learning.

## 2. Methods

### 2.1 Subjects

Male Long Evans rats (n=11, Charles Rivers Laboratories Wilmington, MA) were trained and recorded in the current experiment. Prior to surgery, animals were individually housed under a 12:12 hour light/dark cycle with lights on from 11:30 a.m. to 11:30 p.m. Animals were given ad lib water and restricted food access to maintain their body weight to approximately 330g. Protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH, Publications 865–23) and were approved by the Institutional Animal Care and Use Committee, Rutgers University.

### 2.2 Surgery

Animals were initially anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and given atropine methyl nitrate (10 mg/kg, i.p.) and penicillin G (75,000 U/0.25 ml, i.m.) to prevent respiratory arrest and post-surgical infection. Anesthesia was then maintained by administering ketamine hydrochloride (60 mg/kg, i.p.) as necessary.

Each animal was implanted with an organized microwire array (California Fine Wire, Grover Beach, CA; Microwire technologies) targeting the right nucleus accumbens (0.0-2.8mm AP; 1.6-2.2mm ML; -6.6 mm DV from Bregma). Each microwire array comprised sixteen (2x8) Teflon quad-coated stainless steel microwires (diameter: 50 μm, anteroposterior spacing: 0.35 mm, mediolateral spacing: 0.5 mm) and a connecter strip, which was mounted on the skull and connected the microwires to a harness during neuronal recordings. In addition,

an insulated ground wire was implanted 5.5 mm ventral from skull level in the contralateral striatum to minimize electrical noise.

A one week recovery period followed the surgery, during which animals had ad lib access to food and water. After the recovery period, body weights were maintained around 315g (90% of free feeding) in preparation for training.

**2.3 Procedure**

Experimental procedures were modeled after a conditioning paradigm with sucrose as the unconditioned stimulus developed by Horvitz and colleagues (Choi et al., 2005). Prior to training, all animals were given two days of pre-exposure to 5ml 20% sucrose to eliminate possible novelty effects. Subjects were subsequently trained for 10 consecutive daily sessions in a custom Plexiglas chamber (31.0 cm length, 25.5 cm width, 45.0 cm height), enclosed in a larger sound-attenuating chamber (6249C, Med Associates; St. Albans, VT). Before each training session, animals were connected to the recording harness, through which signals from all 16 microwires were amplified and then relayed through a commutator to a preamp (10X gain, MB Turnkey Design, Hillsborough, NJ) and a filter (500-700X gain, roll off 1.5 dB/octave at 1 kHz and -6 dB/octave at 11 kHz, MB Turnkey Design, Hillsborough, NJ). The amplified and filtered neuronal signal was sampled by a computer with a 50 kHz sampling frequency per recording channel and recorded to hard drive.

Each training session consisted of 28 trials. Each trial initiated with a variable inter-trial interval (VI-54 seconds). Intervals for the VI were selected from a 30 item Fleshler-Hoffman distribution (Fleshler & Hoffman, 1962). At the start of the

ITI, the a sucrose delivery pump (R-E,Razel,Fairfax, VT) was activated for 1 second, delivering ~25 µL sucrose to a retractable dipper (H1405R, Coulbourn; New York, NY). At the end of the ITI, an auditory cue (3.5 kHz, 70 dB) was presented for 500 ms. 250 ms after cue onset, the dipper ascended to the dipper port (3.0 cm wide x 14.5 cm high x 3.0cm deep, centered on the right sidewall of the experimental chamber) so that the sucrose became available to the animal while a light situated in the dipper port became activated simultaneously. The dipper and the light remained activated for 10 seconds, or for 4 seconds from the time the animal made a head entry into the dipper port. Head entries into the dipper port were detected by a set of photocells mounted on the sidewall of the dipper port. The initiation of a new ITI followed the deactivation of the dipper and the light. Neuronal and behavioral timestamps were simultaneously recorded during the experiment and analyzed offline. In addition, video recording of the experimental session was captured at a frame rate of 30 frames/sec. Each individual frame was time stamped by the same software that recorded behavior and neural responses, to ensure synchrony (Datawave Technologies, Loveland, CO).

Following training, animals were deeply anesthetized and each microwire location was lesioned with an anodal current (50 mA, 4 seconds) which was visualized during staining with a 5% potassium ferrocyanide and 10% HCl solution to mark each microwire tip. Animals were intracardinally perfused with saline and 10% formalin phosphate. Brains were extracted and sliced into 50 µm coronal sections and counterstained with a 0.1% solution of Neutral Red. Two

investigators blind to the neuronal data recorded from individual microwires identified the microwire tip placement using the atlas plate that most closely matched the section containing the center of the mark (Paxinos & Watson, 2007). Neuronal data recorded from a microwire were included in the analysis only if the microwire was histologically verified to be in nucleus accumbens core (Figure 1).

### 2.4 Analysis

#### 2.4.1 Video Analysis

Video analysis was performed offline to determine the video frames in which initiation and termination of approaches toward the dipper port occurred. The termination of an approach was identified as the frame in which onset of photobeam interruption occurred or on the first frame of three consecutive frames in which the animal's head remained stable in the bottom of the dipper port but failed to interrupt the photobeam. After the termination of an approach was identified, the video file was played frame by frame backwards in time until the frame in which the initiation of the approach was identified, as follows. During the approach, the animal's movement was required to be constantly directed toward the dipper port. The initiation of the approach (going backward in time) was defined as the last frame in which the animal's movement was towards the dipper port, i.e., the first frame in which movement was directed away from the port. Thus, going forward from that frame, in all consecutive frames, the animal's movement was directed towards the dipper port. If the initiation of an approach could not be identified, that approach was excluded from further analysis.

The approach behaviors were further classified into two categories, cued approaches and non- cued approaches. Cued approach was defined as the first approach behavior initiated after a cue onset and terminated while the dipper was still active. Non-cued approach was defined as an approach behavior that initiated and terminated during the inter-trial interval.

Video analysis of the entire training session was conducted on sessions 1, 2, 3, 4, 5, 7 and 10.

### 2.4.2 Behavioral Analysis

Behavioral variables including the number of missed trials, reaction time to the cue, and latency to head entry were measured and analyzed for every session to assess the effect of training. The number of missed trials was defined as the number of trials in which no head entry to the dipper port occurred during dipper presentation. The reaction time to the cue was defined as the latency to initiate approach following cue presentation, while the latency to head entry was defined as latency to head entry following a cue presentation. Appropriately, reaction time and latency to head entry for a given trial were not calculated if no approach was observed or no head entry was detected during dipper presentation.

Behavioral data (i.e. number of missed trials, reaction time to cue, and latency to head entry) were analyzed with repeated measure ANOVAs with session as a within subjects factor (10 levels of session for number of missed trials and latency to head entry; but 7 levels of session for reaction time to cue, since reaction time to cue could be determined only from sessions in which video analysis was conducted).

Probability of response around cue presentation was also calculated for every animal in each session. Here, a response was defined as the animal's head being inside the dipper port (as measured by the interruption of the photobeam). The probability of response was calculated from 10 seconds before to 10 seconds after cue onset in 1 second bins. The probability of response in individual bins was calculated as the following:

$$Probability\ of\ response\ in\ a\ given\ bin = \frac{number\ of\ trials\ where\ there\ is\ a\ response\ in\ the\ bin}{total\ number\ of\ trials}$$

To compare the probability of response before and after cue presentation, the summation of probabilities in all bins before and after cue presentation was calculated. Repeated measures ANOVAs were conducted, with the sum of probability as the dependent variable, time period (before or after cue onset, 2 levels) and sessions (sessions 1 through 10, 10 levels) as the independent variable.

Individual differences in learning were examined by graphic analysis initially. The response latency to the cue during individual trials was plotted across time. When the animal did not make a response during a trial, response latency was plotted as 10 sec (dipper offset). It was observed that most animals exhibited a transition in response latency that followed a similar pattern: early in training, response latency fluctuated around a relatively high level, which rapidly dropped at some point during initial training and fluctuated around a low level for the remaining training days. Different animals displayed this drop in response latency after different trials. Inflection point of learning was defined as the following and identified for individual animals to capture the trial where the latency drop

finished: (1) the trial was a local minimum on the latency graph (2) the trial had a latency that was shorter than the 25$^{th}$ percentile of all latencies (3) the trial had a latency that is smaller than the median of all the latencies in the last session (session 10). For individual animals, each session before the inflection point was designated as a pre-inflection point session, whereas each session(s) after the inflection point was designated as post-inflection point session(s). The session that the inflection point fell in were determined as a pre-inflection session if the inflection point happened in the latter half of the session, and were determined as a post-inflection session otherwise. The pre-inflection sessions were considered as the sessions when the animal had not fully learned the cue-response-reward association, whereas the post-inflection sessions were considered as the sessions where the animals had learned the cue-response-reward association. This customized dichotomization was applied to analysis of neural activities described below, so that the potential differences in neural activity caused by the individual differences in learning could be appropriately addressed.

### 2.4.3 Neuronal Analysis

#### 2.4.3.1 Spike Sorting

Neuronal data recorded from the microwires that were localized to the accumbens core were played back offline and sorted by the shape of the waveform (Datawave Technology, CO). The purpose of spike sorting was to eliminate electronic noise and separate multiple units in instances where more than one neuron was recorded on a single microwire. The following waveform parameters were used in spike sorting: peak time, peak amplitude, spike time,

spike height, principle components and two custom voltage cursors. Neuronal signals were considered recorded from one single neuron when the following criteria were met: (1) A signal-to-noise ratio that is greater than 2:1 (2) signals exhibit similar shapes, and have similar values in all waveform parameters. (3) auto-correlation revealed a minimum inter-spike interval (ISI) ≥ 2 ms (natural refractory period). A Cross-correlation was performed if several waveform profiles were detected in the recording from one microwire. If cross-correlation revealed at least one instance of ISI smaller than or equal to 1ms, the signals were considered to originate from different neurons (whose auto-correlations each showed zero spikes within 2 ms). Otherwise, the signals were combined and considered originating from one neuron. The timestamps of the sorted action potentials were saved for future neuronal analysis.

Signal were considered to be recorded from the same neuron across sessions when the following criterions were met: (1) Waveforms were recorded from the same microwire (2) waveforms had similar shapes: the correlation between the average waveforms (average waveform voltages during the spike, sampled at 35 time points at 50 kHz) from one session to the next was greater than 0.9, (3) waveforms had similar parameters: difference in spike height from one session to the next was less than 20 %, and the difference in peak time from one session to the next was less than 0.04 ms. See Figure 2 for the waveform of an example neuron recorded across 10 sessions.

**2.4.3.2 Calculating Neural Activity during Specific Behavioral Events**

To characterize the sensitivity of accumbens core neurons to reward-related events, firing rate during different reward-related events were computed and compared to a baseline firing rate (defined below). Firing rate (spikes/second) during all reward related events was calculated on a trial by trial basis, i.e. number of spikes during a reward related event divided by the duration of that particular event. The following reward related events were examined: cue, cued approach, non-cued approach, reward consumption and reward seeking. Firing rate in response to the cue was examined in a time window starting from the onset of the cue and ending at either the offset of the cue or the initiation of an approach, whichever occurred earlier. Cued approaches and non-cued approaches were determined from video analysis as described above. Only approaches with durations longer than 166 ms (five video frames) were analyzed. The time window for analyzing reward consumption initiated at the first head entry after the dipper activation and terminated at the corresponding head exit or the dipper deactivation, whichever occurred first. The time window for analyzing reward seeking initiated at the head entry and terminated at the corresponding head exit.

After the trial by trial neural activity data were obtained, cued approach and non-cued approach within the same recording session were matched for duration to minimize any contribution of motoric differences to potential firing differences between cued approach and non-cued approach. A cued approach was considered a match with a non-cued approach if their duration ratio ($\frac{latency\ of\ cued\ approach}{latency\ of\ non-cued\ approach}$) was between 0.8 and 1.2. A greedy algorithm with

randomization was developed to optimize the quality of the matching: for every cued approach in a given recording session, find the non-cued approach that satisfies the matching criterion with a duration ratio closest to one. The cued approach and the selected non-cued approach formed a matched pair. The selected cued approach and non-cued approach were then removed from the candidate list for matching so each approach was only used once. For a given cued approach, if no non-cued approach satisfied the matching criterion, the cued approach was removed. One run of the algorithm would result in a list of matched pairs of cued approach and non-cued approach. The summation of the absolute differences between the latencies of each matched pair was calculated as a measurement of matching quality. This algorithm is sensitive to the order of the list containing cued approach latencies. In certain cases, the order of the latency list might lead to sub-optimal matching. Therefore, the algorithm was executed five times for every recording session with a different random permutation of the sequence of cued approaches, resulting in five lists of matched pairs. The list of matched pairs with the best matching quality (i.e. smallest summation of absolute difference) was selected as the final list of matched pairs.

Baseline firing rate was computed on a trial by trial basis, i.e. for every occurrence of a reward related event. The duration of the baseline was always the same as its corresponding behavioral event. The baseline of cue reactivity was computed in a time window with the same duration (e.g., *t sec*) as the time window used for computing cue-evoked firing in the same trial, but ending at the

cue onset, starting at *-t sec* before cue onset (i.e. the mirror image of the time window for calculating cue reactivity). For cued approach, non-cued approach, consumption and seeking, baseline firing rates were calculated in the following fashion: (1) determine the sampling window of the baseline: the sampling window of the baseline was a period of time that preceded the occurrence of a reward related event and was free of any other reward related event (e.g., approach, head entry). More specifically, the end of a baseline sampling window for a specific reward related event was defined as the time when the initiation of an approach or a cue onset occurred, whichever occurred first. The start of a baseline sampling window was defined as the time of the head exit from the dipper port directly preceding the end of the baseline sampling window. (2) sample and compute baseline firing rate for a given trial: baseline sampling window was divided into 33 ms intervals (same duration as a video frame). $N$ intervals were randomly drawn from all the intervals,

with $N = \frac{Duration\ of\ the\ reward\ related\ event\ in\ question}{Duration\ of\ the\ interval}$. The baseline firing rate was calculated as the following: $FR_{baseline} = \frac{\sum_{n=1}^{N} Number\ of\ spikes\ in\ interval\ n}{Duration\ of\ the\ interval * N}$. Baseline firing rate was considered missing if there was less than $N$ intervals available in the baseline sampling window. Trials with either missing event firing rate or missing baseline firing rate were omitted from further analysis.

In addition to firing rate, the latency to first spike was also calculated as a separate measure for reactivity to the cue. Latency of the first spike to the cue was calculated as the following: (1) find the time $t_{spike}$, at which the first spike occurred after cue onset (the time of cue onset was designated as $t_{cue\_onset}$) and

before the cue offset ($t_{cue\_offset}$) or the approach onset ($t_{approach\_onset}$), whichever came first. Formally, the time window to search for the first spike was: $[t_{cue\_onset},\ \min(t_{cue\_offset}, t_{approach\_onset})]$. If a spike did not occur in the time window, the measurement was considered missing for that trial. (2) Latency to the first spike was calculated using the following formula: $latency\ to\ first\ spike\ after\ the\ cue = t_{spike} - t_{cue\_onset}$. The latency of first spike to the cue was compared to the latency of first spike to the baseline onset, defined as the following: $t_{cue\_onset} - \min(t_{cue\_offset}, t_{approach\_onset})$.

**2.4.3.3 Modeling firing rate during specific behavioral events**

To explore the sensitivity of neurons to the different behavioral event at different stages of learning, generalized linear mixed models (Glimmix models, (Breslow & Clayton, 1993)) were constructed. Separate models were constructed to model the following neural activities: (1) firing rate related to cue-reactivity (2) firing rate during approach (in this model, cued approach and non-cued approach within the same recording session were matched for duration. Firing rates during matched approaches were used in order to eliminate motoric differences between cued versus non-cued approaches.) (3) firing rate during reward consumption or reward seeking (4) latency of first spike to the cue (see results section of lists of independent variables considered for different models). For all models with firing rates as the dependent variable, 1 was added to the original firing rates (to handle zero inflation) and modeled with gamma distribution with log link. For the model with latency of first spike to the cue as the dependent variable, the latency was modeled with gamma distribution with a log link. For all models, laplace

method was used for the likelihood estimation with $10^{-8}$as the convergence criterion. Sandwich-MBN method was used for fixed effect standard error adjustments. To avoid unnecessary comparisons and balance between type I and type II error rate, post hoc comparisons were grouped in to $M$ groups ($M$ is the number of variables exhibiting significant interaction; see Results section for designs of post hoc comparisons for specific models). Holm-Sidak correction for multiple comparisons was applied within each group of post hoc comparisons.

#### 2.4.3.4 Quantifying the Reactivity of Individual Neurons to Specific Behavioral Events

To obtain a quantitative measure of sensitivity for individual neurons during different reward related events, change scores were calculated as following: $\frac{FR_{event}-FR_{baseline}}{FR_{event}+FR_{baseline}}$ for every neuron, individual events. Change scores were calculated for a given event on a trial by trial basis. Then, change scores for that event were averaged within session for individual neurons recorded during a specific session. Change scores were calculated for five behavioral events: (1) cue, (2) cued approach, (3) reward consumption, (4) non-cued approach, and (5) reward seeking. Distributions of change score pre- and post-inflection point were plotted for individual behavior events. To test whether the distribution of change scores during a specific event was indeed induced by that event, but not due to random fluctuation of spontaneous neural activity, neural activity with the same duration of the event in question was randomly sampled from the same session. Change scores were calculated as: $\frac{FR_{random\_sample}-FR_{baseline}}{FR_{random\_sample}+FR_{baseline}}$. The distribution of

the event change scores was compared against the distribution of the change score of the randomly sampled activity with Kolmogorov–Smirnov test.

#### 3.4.3.5 Stability of Neural Responses across Sessions

To investigate whether neurons respond to individual events similarly across sessions, correlations were calculated for pairs of sessions. Correlations were considered significant if $p < 0.05$ after FDR correction for multiple comparisons (Verhoeven et al., 2005). The correlation coefficients were visualized in heat maps. Cue, cued approach, consumption and seeking were investigated, but not non-cued approach, because previous analysis showed that core neurons did not exhibit responsiveness to non-cued approaches.

## 3. Results

### 3.1 Behavioral Results

Three behavioral measures were taken to evaluate learning: (1) number of missed trials in a given session, (2) head entry latency, defined as the time difference between first head entry after the cue onset and the cue onset in a given trial, (3) reaction time to the cue, defined as the time difference between the initiation of a cued approach and the cue onset in a given trial. Repeated measures ANOVAs, with session as the independent variable, were implemented to examine the effect of training.

In order to quantify learning over days, the number of missed trials (Fig. 3 A) and average head entry latency (Fig. 3 B) in a given session were calculated for every animal and session. A significant main effect of session was found for both the number of missed trials and average head entry latency ($F(9,90)=12.545$, $p<0.01$ and $F(9, 90)=23.561$, $p<0.01$ respectively). Post hoc analysis revealed a significant quadratic trend for the number of missed trials ($F(1,10)=23.14$, $p=0.01$) with a monotonic decrease and asymptote from session 1 (13.18 ±2.27, Mean±SEM) to session 10 (0.73±0.24). Similarly, for head entry latency, post hoc analysis revealed a significant quadratic trend ($F(1,10)=32.61$, $p<0.01$) with a monotonic decrease and asymptote from session 1 (4.15±1.00 s) to session 10 (1.82±0.61 s).

The timestamps of cued approach onset were determined from video analysis. Therefore, reaction time to the cue (Figure 3 C), which depends on the cued approach onset time, was only calculated for animals and sessions (sessions 1

to 5, 7 and 10) where the video analysis was conducted. A repeated measures ANOVA with average reaction time to the cue as the dependent variable and session as the independent variable was implemented to examine change in reaction time through training. Reaction time to the cue decreased as training proceeded over sessions, $F(6,48)=10.82$, $p=0.02$, which was a significant linear trend $F(1,8)=18.27$,$p<0.003$. This result demonstrates that the reaction time to the cue significantly decreased as animals learned the cue-reward association, with an average reaction time of 3.37±0.73 s during session 1 and an average reaction time of 0.68±0.12 s during session 10.

In order to rule out the alternative explanation that these behavioral changes over sessions was due to nonspecific increased responding across training, the probability of responding around cue onset was examined. Figure 4 shows the average probability of responding across all animals during individual sessions in a 20 second window around the cue onset. The average probability of response was low and steady before cue onset and started to increase immediately following cue onset (Figure 4, A). To quantify this observation, the area under the response probability curve was calculated before and after the cue for individual animals / individual sessions. A repeated measures ANOVA was conducted, with time period (2 levels, before the cue or after the cue) and session (10 levels) as the independent variables and area under the response probability curve as the dependent variable. The model revealed significant main effects of time point ($F(1,10)=307.72$, $p<0.01$) and session ($F(9,90)=5.71$, $p<0.01$), and a significant interaction between time point and session ($F(9,90)=7.83$, $p<0.01$). Post hoc

analysis was conducted to compare the difference between the change of response probability before and after the cue across sessions. The post hoc analysis revealed a significant quadratic trend between the difference of the change across sessions before and after the cue ($F(1,10)=40.258$, $p<0.01$). This effect is visualized in Figure 4B, in which the pre-cue probability of response remained low and stable across sessions, whereas the post-cue probability of response increased and asymptoted across sessions. These results demonstrate that animals learned the cue-reward association over training sessions as they initiated responses more often with shorter latency selectively after the onset of the cue as a result of training.

In order to examine individual differences in learning, response latency for every trial was plotted across time. Visual inspection showed dissimilarities in the rate of behavioral change (see error bars in Figure 3 C, and data from representative animals in Figure 5). Therefore, an inflection point was determined for every animal as an operational definition designating sessions before versus after learning had occurred. The median inflection point was session 3 and ranged from session 1 to session 7. The average number of missed trials was 10.56±1.48 before the inflection point, and 1.39±0.31 after the inflection point. The average response latency was 3.28±0.10 s before the inflection point and 2.14±0.07 s after the inflection point. And the average reaction time was 2.33±0.10 s before the inflection point and 1.23±0.07 s after the inflection point. Therefore, the neural analysis below was based on each animal's inflection point to better assess the relationship between learning and neural responses.

## 3.2 Neural Results

Out of 11 animals recorded, 9 animals exhibited single-units from the accumbens core. A total of 56 accumbens core neurons were recorded in one or more sessions resulting in 286 neuron-sessions.

### 3.2.1 Neural activity during specific behavioral events

#### 3.2.1.1 Neural activity during the cue

To assess the population response of core neurons during the reward-related cue, a three level Glimmix model was constructed to model the raw firing rate in response to cue, with firing rate (spikes/second) as the independent variable. The dependent variables were: (1) response (whether the animal responded to obtain the reward after the cue or not, in two levels), (2) event (cue or baseline, in two levels), and (3) inflection point (before or after the inflection point indicating whether learning had occurred, in two levels).

We first examined changes in firing rate following cue presentation. The model revealed a significant main effect of event ($F(1,15723)=16.35, p<0.001$), with a higher firing rate during the cue (1.31±0.13 spikes/sec) compare to that during the baseline (1.10±0.12 spikes/sec), $p<0.001$. Other main effects and all interaction terms were not significant (Figure 9A). This result suggests that cue presentations increase core firing rates independent of learning.

Besides firing rate during the cue, the latency to first spike after the cue onset was examined as a separate measure of neural excitability during cue presentation. Latency to the first spike after the cue onset was compared to the latency to the first spike after baseline onset. A three level Glimmix model was

constructed to model latency to the first spike. The dependent variables were: (1) response (whether the animal responded to obtain the reward after the cue or not, in two levels), (2) event (cue or baseline, in two levels), and (3) inflection point (before or after the inflection point indicating learning had occurred, in two levels).

The Model revealed a significant three way interaction between response, event and inflection point ($F(1,6234)=4.44$, $p=0.035$), and a two way interaction between response and event ($F(1,6234)=4.53$, $p=0.033$). Three sets of post hoc comparisons were conducted to examine the three-way interaction: (1) The first set of post hoc comparisons compared the latency to first spike after cue onset vs. baseline onset across four different combinations of levels of inflection point and response. The four combinations were: before inflection point when the animal did not respond to the cue (pre-inflection miss), before inflection point when the animal responded to the cue (pre-inflection hit), after inflection point when the animal did not respond to the cue (post-inflection miss), after inflection point when the animal did respond to the cue (post-inflection hit). The post hoc analysis revealed a significant difference between the latency of first spike to cue (198.3±4.3 ms) in comparison to that of the baseline (217.19±5.0 ms) for post-inflection hits. However, there were no statistically significant differences between cue and baseline for the other three cases (Figure 9B). (2) The second set of post hoc comparisons compared latency to first spike between trials when the animal responded versus did not respond to the cue, across four different combinations of levels of inflection point and event. The post hoc comparison

failed to reveal any significant differences. (3) The third set of post hoc comparisons compared latency to first spike before and after inflection point across four different combinations of levels of event and response. The post hoc comparison failed to reveal any significant difference. The fact that the difference between cue and baseline firing existed only when the animal responded to the cue after the inflection point indicates that core neurons may process reward-related cues differently before versus after learning, and that this processing may be influential on trials in which the animal responds for reward.

#### 3.2.1.2 Neural Activity during Approach

We next examined changes in firing rate during the reward-directed approach. To assess the general activity of all core neurons, a three level Glimmix model was constructed to model the firing rate during approach behavior, with firing rate (spikes/second) as the independent variable. The dependent variables were: (1) event (approach or baseline, in two levels), (2) type of approach (cued approach or none-cued approach, in two levels), and (3) inflection point (before or after inflection point indicating learning had occurred, in two levels).

The model revealed a significant main effect of event ($F(1, 16231)=4.49$, $p = 0.034$) and a significant event x approach interaction ($F(1, 16231)=7.29$, $p <0.01$), indicating the change of firing rate from baseline during the reward-directed approach is different between cued versus non-cued approach behaviors. Two sets of planned comparisons were devised to pinpoint the source of this difference. The first set of planned comparisons compared the difference in firing rate during baseline versus approach behavior for (1) cued approach and

(2) non-cued approach. The second set of comparisons compared the difference in firing rate between (3) cued approach baseline and non-cued approach baseline and (4) cued approach and non-cued approach. Out of the 4 comparisons, only the difference between baseline and cued approach firing rates was significant ($p<0.01$, $t(16231) = -3.28$), indicating an increased firing rate selectively during cued approach (1.21±0.12 spikes/sec) compared to baseline (1.02±0.12 spikes/sec). Non-cued approach firing rates (1.11±0.13 spikes/sec) were not significantly different from baseline (1.10±0.13, Figure 10). This result indicates that core neurons specifically increase firing rates during cued, but not non-cued approach behaviors.

Because behavioral data indicated that cued approach became a conditioned response post-inflection after the animal acquired the association between the cue and the sucrose reward, it was hypothesized that the differences in firing rate between cued approach and baseline might be different pre- vs. post-inflection point. Two sets of two planned comparisons were conducted to test this hypothesis. The first set of planned comparisons compared the firing rate difference between baseline and cued approach before versus after inflection point. The second set of planned comparisons compared baseline firing rate before and after inflection point, and cued-approach firing rate before and after inflection point. The tests revealed significant differences between baseline firing rate and cued approach firing rate both before ($t(16231)=-2.56$, $p<0.021$) and after inflection point ($t(16231)=-2.25$, $p=0.025$). However, there was no statistically significant difference between baseline firing rate before and after

inflection point, or between cued approach firing rate before and after inflection point. This result indicates that the difference in firing rate between cued approach and baseline remain constant before and after inflection point, as did the firing rates during baseline as well as cued approach. Firing during cued approach was similarly enhanced pre and post inflection point, indicating that the increased activity in core during cued approaches was independent of whether the animal had reached a behavioral criterion (inflection point in this case).

#### 3.2.1.3 Neural Activity during Consumption and Seeking

A three level Glimmix model was constructed to model the average firing rate during consumption and seeking in comparison to baseline firing rate, with firing rate (spike/second) as the independent variable. The dependent variables were: (1) event (baseline or consumption/seeking, two levels), (2) consumption/seeking (two levels, consumption or seeking), and (3) inflection point (before or after the inflection point indicating learning had occurred, two levels).

We next examined changes in firing during periods in which the animal was inside the reward receptacle. During cued trials, animals licked the sucrose delivery spout (consumption) and during uncued trials, the sucrose was unavailable to animals (seeking). The model revealed a main effect of event ($F(1,20154)= 4.07$, $p<0.05$) and a two-way interaction of event x consumption/seeking ($F(1,20154)=4.02$, $p<0.05$). The significant two-way interaction suggests that the differences in firing rate during baseline and event was different for consumption and seeking. In order to examine this possibility, two sets of two planned comparisons were conducted. The first set compared the

difference between baseline firing rate and event firing rate for (1) consumption and (2) seeking. The second set compared the difference between (3) baseline firing rate for consumption versus that of seeking; (4) event firing rate for consumption versus that of seeking. Among the four tests mentioned above, the post hoc analysis revealed only one significant difference ($t(20154)=2.42$, $p=0.031$) between the firing rates during baseline (1.02±0.13 spikes/sec) and consumption (0.90±0.12 spikes/sec, Figure 11). This result shows that core neurons significantly decrease their firing rate specifically during reward consumption compared to baseline.

### 3.2.2 Heterogeneity of Neural Responses

We next assessed the reactivity of individual neurons to specific behavioral events during individual sessions. The possible range of change scores was from -1 to 1. A change score of 0 indicates no change in firing rate from baseline to a given behavioral event. The larger the absolute value of the change score, the greater the change. The sign of the change score indicates the direction of change: negative values indicate decreases from baseline, whereas positive values indicate increases from baseline. The distributions of change scores for different behavioral events were constructed pre- and post-inflection point (Figure 12). The distribution of change scores for the cue, cued approach, and reward consumption were significantly different from their corresponding distributions generated from random sampling (cue pre-inflection, $p < 0.05$, $D =0.194$, cue post-inflection, $p < 0.01$, $D = 0.230$; cued approach pre-inflection, $p < 0.05$, $D=0.185$, cued approach post-inflection, $p < 0.01$, $D=0.2079$; consumption pre-

inflection, $p < 0.01$, $D = 0.4259$, consumption post-inflection, $p < 0.01$, $D = 0.4045$; seeking, pre-inflection, $p < 0.05$. $D = 0.194$, seeking post-inflection, $p < 0.01$, $D=0.24$). However, the distribution of the change scores for the non-cued approach was not significantly different from the corresponding random sample (which would correspond to random fluctuation in neural activity). These results indicate that, both before and after inflection point, core neurons exhibit significantly different changes from baseline firing rate specifically during cue presentation, cued approach, reward consumption, and reward seeking, but not non-cued approach.

### 3.2.3 Stability of Neural Responses across Sessions

The session-to-session stability of neural responses during individual events was assessed by computing the Pearson correlation for the change scores of individual neurons between pairs of sessions. Strong and positive correlation coefficients between temporally close sessions indicates good stability, i.e. neurons respond similarly to the same event from one session to the next. The results are illustrated in Figure 13. The neural responses during cue presentation, cued approach and reward seeking were not consistently stable across session, indicated by weak correlation coefficients and a large proportion of non-significant correlations. In contrast, the neural responses during reward consumption displayed high stability, as indicated by moderate to strong correlation coefficients and high proportion of statistically significant correlations. These results indicate that individual core neurons consistently responded to reward consumption across training sessions, exhibiting a general inhibition.

However, individual core neurons did not consistently respond to other reward related events (e.g. cue presentation or cued approach) from session to session, indicating that the general neural excitation observed during these events was mediated by different neurons from session to session.

# 4. Discussion

## 4.1 Individual Differences in Learning

Averaged across all animals, the behavioral metrics that measured learning (i.e. number of missed trials, latency to response and reaction time to cue) decreased as training progressed, indicative of learning. However, the gradual decrease of these behavioral metrics over training did not reflect the behavior of individual animals, and was an effect of group averaging. As shown in Figure 5, individual animals displayed rather abrupt drops in response latency, generally spanning a dozen trials. Further, different animals displayed this drop in response latency at different trials and days, spanning a wide range. Similar results have been described by (Gallistel et al., 2004). The abrupt change in individual learning curves is generally incompatible with classical theory on associative learning, which is established based on learning curves averaged across a group of animals. The theoretical impact of this observation is discussed in detail by Gallistel, 2004.To assess the change in neural activities through the progression of conditioning, it is reasonable to anchor neural activities to individual animals' behavior rather than the behavior of the group of animals. Given the abruptness of the change in behavior, it is appropriate to dichotomize the training of individual animals to two periods: pre-inflection point and post-inflection point (see definition in method section). Neural analysis was conducted based on the customized dichotomization for individual animals, rather than over individual sessions. The latter does not capture learning as an experience unique to different animals. Analyzing neural data after a conditional response has

stabilized is a common practice in behavioral electrophysiology. However, few studies have investigated neural activity before conditioning has stabilized, despite its importance. The current study examined and compared the neural activity in the core through the whole course of conditioning.

### 4.2 Neural activity pre- and post-inflection point

#### 4.2.1 Neural Activity during Cue Presentation

Dopamine neurons of the VTA, which strongly innervate the accumbens, acquire reactivity to a reward-related cue as animals learn a cue-reward relationship (Schultz, 2002). Similar patterned changes in dopamine release occur in the accumbens through conditioning, though sub-regions of the accumbens were not distinguished (M. F. Roitman et al., 2004a). The present study observed increased firing rates in accumbens core neurons during cue presentation both pre- and post-inflection point. Similar unconditioned firing rate changes in response to an auditory stimulus have been observed in accumbens core (Ghitza et al., 2004). The source of the increased core firing rates following cue presentation is likely from several brain regions. In addition to cue-related dopamine projected to core, chemical inactivation studies suggest that BLA input is critical for cue-related excitation in the core (Jones et al., 2010). Cue reactive neurons have been observed in the amygdala both before and after fear conditioning was acquired (Quirk et al., 1997). Moreover, it was observed that the neural response latency to the cue was significantly shorter after fear conditioning was acquired (Quirk et al., 1997). The current study revealed a similar decreased latency to fire in core neurons following cue presentation after

learning. Therefore, it is possible that the elevated firing observed in the core was at least in part driven by amygdaloid input, but may also have been facilitated by enhanced dopamine signaling. .

### 4.2.2 Neural Activity during Approach

The accumbens core is believed to be a critical brain region in promoting cue-induced approach (Ambroggi et al., 2011; Phillips et al., 2003; M. F. Roitman et al., 2004a; Wakabayashi et al., 2004). In the current study, the firing rate during cued-approach was compared to baseline and also to firing rate during non-cued approach. Cued approach behavior and non-cued approach behavior were matched in duration so that motoric differences were unlikely to account for any observed neuronal differences. An increased firing rate during cued-approach was found in accumbens core neurons compared to baseline. The difference in neural activity from baseline to cued-approach was not caused by motoric differences, since there was no difference between the activities during non-cued approach compared to baseline. This result indicates that cued approach is processed by core neurons differently than non-cued approach. Indeed, the special nature of this core activity is illustrated by the finding that similar magnitudes of increased firing rates pre- and post- inflection point were observed during cued approach. That is, whenever the animal responded to the cue, regardless of the probability of responding in that session, core neurons exhibited increased firing rates.

The similar magnitude of excitation pre and post- inflection point in the core is likely mediated by the interaction of different neurotransmitter systems. It has

been shown previously that co-activation of D1 and NMDA receptors in the accumbens core was required for acquisition of conditioned responses to reward related cues, whereas neither D1 nor NMDA receptor antagonists could disrupt the expression of conditioned responses after they were acquired (Choi et al., 2011; Kelley, 2004; Jon Horvitz, personal communication). Interestingly, we observed no phasic change in neural activity during non-cued approaches. However, it has been shown that D1 receptor blockade was effective in reducing the number of non-cued approaches in this paradigm (Choi et al., 2011). Although there was no prominent change in core firing rates during non-cued approaches, a certain level of core activation (i.e. spontaneous firing) which could be reduced by dopamine receptor blockade, may be required for the initiation of spontaneous behaviors (Baldo et al., 2002).

### 4.2.3 Neural Activity during Consumption and Seeking

The present decrease from baseline activity in accumbens core neurons during reward consumption, is consistent with previous studies that showed decreased firing during consumption compared to approach (Krause et al., 2010; Taha & Fields, 2005). Moreover, comparing neural activity pre- and post-inflection point indicated that the neural activity in core during consumption did not change as a function of learning. It has been reported previously that the majority of core neurons showed unconditioned reactivity to oral infusions of tastants (M. F. Roitman et al., 2005), and a subpopulation of neurons displayed sensitivity to palatability and relative palatability (Taha & Fields, 2005). The present study showed that when the animal was inside the reward delivery port without the

reward delivered, the average firing rate during seeking behavior was not significantly different from baseline. This indicates that environmental cues such as the reward delivery port itself have a minimal effect on average neural activity in the core. Taken together, these results indicate that the decrease in core firing during consumption may reflect the rewarding property of sucrose itself.

In contrast to these accumbens patterns, dopamine neuron activity in the VTA, a key afferent to the accumbens, changes over the course of conditioning during reward consumption (Schultz, 2002). Increased dopamine neuron activity during reward consumption was observed early in conditioning but gradually decreased in magnitude and eventually disappeared. Dopamine release in the accumbens follows the same pattern through conditioning (Day et al., 2007). However, the accumbens, being one of the targets of the VTA dopamine neurons, continues responding during reward consumption after the acquisition of cue-reward relationships (Krause et al., 2010; M. F. Roitman et al., 2005; present data). This suggests that the neural activity during reward consumption might be modulated by factors other than or in addition to dopamine. One potential influence is the endogenous opioid system. Intra-accumbal microinjection of μ-opioid agonist was shown to increase feeding behavior (Kelley et al., 2002; Woolley et al., 2006), whereas a δ-opioid antagonist was shown to increase feeding (Kelley et al., 1996). The convergence in the accumbens of dopaminergic inputs regarding the learned properties of the reward, and opioid signaling regarding the hedonic value of the reward, may enable modification of the cue-reward relationship in order to guide goal-directed behaviors.

Firing rate changes of individual neurons were of smaller magnitude than those often reported in the accumbens literature. This is likely due to several aspects of the present design. First, analyses of firing were divided into smaller time periods corresponding to specific behaviors, such as cue processing versus approach. Second, rather than highlight “responsive” neurons, the objective was to draw inferences about the accumbens population as a whole by including the entire sample in analyses. Third, each session contained 28 trials that were displayed in any individual raster/PETH, compared to much larger numbers of trials in other studies, which may enhance signal to background.

**4.3 Heterogeneity of Core Activity**

Individual Nacc core neurons displayed a certain amount of daily variability in their activity, both in general (as shown by the distribution of the change scores for the firing rate from random sampling) and during specific reward related events. The distributions of change in neural activity during certain reward related events (cue, cued approach, reward consumption, and seeking) were significantly different from that of random fluctuation. A heterogeneous response pattern (increase, decrease, or no change from baseline during specific events) has been reported previously in studies involving electrical stimulation, receptor activation by an agonist, addictive drug self-administration and natural reward paradigms (Carelli & Deadwyler, 1994; Carelli & Wondolowski, 2006; Chang et al., 1994; Ghitza et al., 2004; Jones et al., 2010; Nicola et al., 2004). The functional heterogeneity may be a result of structural heterogeneity. Although the accumbens is dichotomized into core and shell, the distribution of afferents

between and within each subregion changes along several gradients. For example, from ventral to dorsal, the subiculum of the hippocampal formation projects to the accumbens medially to laterally, respectively. Similarly, BLA and other hippocampal projections into the accumbens show mediolateral topographies. Hippocampal inputs tend to be restricted to shell and the medial and rostrolateral parts of the core (L. Heimer et al., 1997). Moreover, anterograde tracer injections into the input structure of accumbens in combination with retrograde tracer injection into target structures of accumbens efferents revealed that clusters of output neurons receive specific sets of inputs (Pennartz et al., 1994). This evidence supports the hypothesis of functionally distinct neural ensembles in the accumbens, which may result in the observed heterogeneous activity.

**4.4 Stability of Neural Responses across Sessions**

Our goal was to track individual core neurons over the entire course of learning. This enabled an assessment of stability of neuronal responses. For instance do individual neurons respond similarly to a reward related event every day? The stability of responses was examined for four events: cue presentation, cued approach, consumption and seeking. Correlations of neural activity between pairs of sessions were computed as a measure of stability. Out of a total of 84 correlation tests conducted, 14 correlations were statistically significant at the level of $p < 0.05$ after FDR correction. Of the 14 significant correlations, 12 cases were neural activity during consumption. Further, the correlation for neural activity during consumption was significant between all pairs of consecutive

sessions. This highly nonrandom allocation of significant correlations highlights the strong stability of neural activity during consumption. The high stability during consumption is consistent with the hypothesis that accumbal neurons may have innate tuning to different tastants or represent their value (M. F. Roitman et al., 2005) The strong stability of neural activity during consumption reaffirmed our confidence in being able to track individual neurons across multiple sessions. It is highly unlikely that recording different neurons across session would lead to such strong correlations over multiple pairs of sessions. In fact, the significance test for correlation is conducted by comparing the correlation coefficient computed from paired neural activity from two sessions to a distribution of correlation coefficients, which is generated by disrupting the pairing in the data between sessions. Disrupting the pairing in the data, in this specific scenario, is effectually equivalent to assigning a new neuron to the same micro-wire from one session to the next. The significant correlations indicate that the relationship of neural activity between consecutive sessions during consumption is highly unlikely to be present if a different neuron had been recorded on the next session. The present findings extend the well known trial-to-trial variability in a single neuron's activity, such as in medium spiny neurons (Prokopenko et al., 2004), by demonstrating session-to-session variability in a given neuron's firing pattern.

The reason for the instability of responses during cue and cued approach might be multifaceted. When analyzing the neural stability during cue presentation, we pooled trails in which animals made cued responses, together with trials in which animals did not respond. The potential differences in neural processing in the two

types of trials might lead to the observed instability. Further, it is likely that the animals' experience with the cue-reward relationship might affect neural responses during cue presentation. Trials preceded by a trial in which the animal experienced the reward might have differential firing activity in comparison to trials preceded by a trial in which the animal missed the opportunity to obtain the reward. To address both issues, further analyses will be done to evaluate the neural activity and stability taking into account the behavioral responses in the present and preceding trials.

More generally, the observed lack of stability might be an artifact of insufficient experimental design. Unlike the primary sensory/motor areas, the signal that reaches the accumbens has been processed previously in multiple regions and is further processed by multiple downstream regions. It is difficult to pinpoint the effective external stimulus that elicits a neural response or the behavioral output triggered by the neural response. It is likely that a neuron in accumbens core may respond to a specific aspect of the cue in combination with a specific internal state. An experimenter observing the neural activity of this specific neuron during the same cue might reach the conclusion that the neuron is responding to the cue in an unpredictable fashion, lacking knowledge of the internal state. Alternatively specific representations (external stimulus or internal state or behavioral output) might not be encoded by the activities of individual neurons, but by the activity of a group of neurons. If this is the case, as long as the group of neurons responsible for encoding a specific representation is responding consistently during a reward related event, individual neurons in the

group do not have to respond consistently. The substantial convergence in the projection of accumbal medium spiny neurons into ventral pallidum lends support to this hypothesis.

The present study demonstrated several key advances in the analysis of neural activities in the accumbens core. Firstly, different from previous studies, the current study established careful motor control when analyzing the neural activity during cue presentation and approaches, such that the observed differences in neural activity can be correctly attributed to processing of the cue instead of motoric effects. Secondly, video analysis was used to ensure that all comparisons of firing were performed between behavioral events that were matched with respect to motor behavior, to minimize any differences in firing related to movement alone. Thirdly, analyses were oriented toward the activity of the population of accumbens core neurons, rather than the activity of only "responsive" neurons, providing a different perspective from previous studies, which may be meaningful since regions downstream of the core are likely processing the activity of the whole collection of core neurons. Last but not least, individual neurons were tracked across sessions, producing some unexpected results. To our best knowledge, this is the first study that examined neural stability in the accumbens core, revealing that conditioned behaviors involving the core may be accomplished by different neurons from within the population from session to session.

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Fig.1

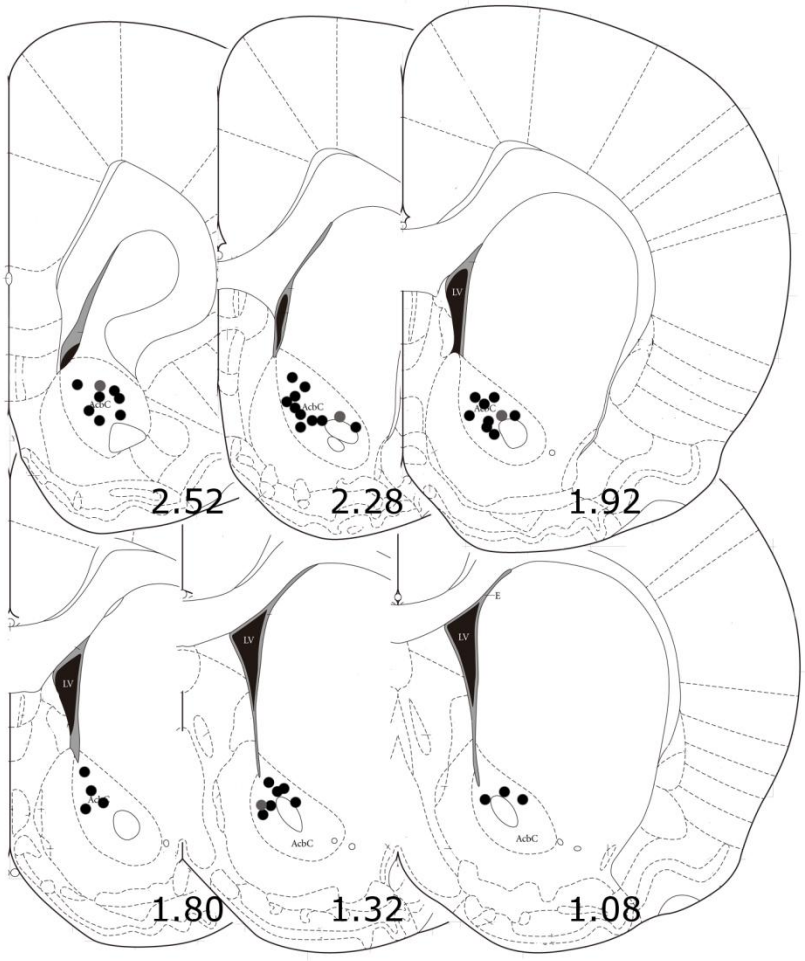


Figure 1: Histology. Location of neurons recorded in this study, histologically verified to be in the accumbens core. Black dots represent one single neuron recorded; grey dots represent two or more single neurons recorded at that location. Six coronal sections of striatum are shown with the anterior posterior distance from bregma labeled.

Fig. 2

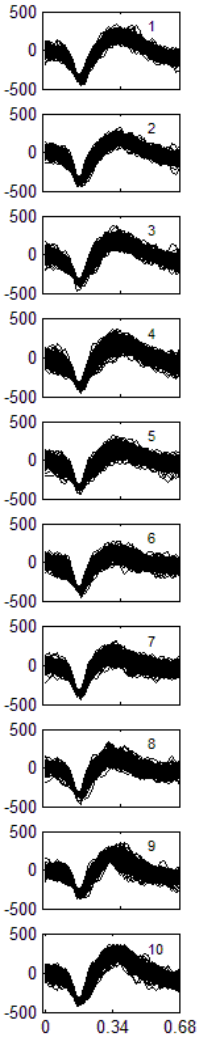


Figure 2: Representive waveforms recorded from the same neuron across session. The waveforms bear resemblelance across session. Number in individual subfigure indicates the session from which the waveform was recorded.

Fig.3

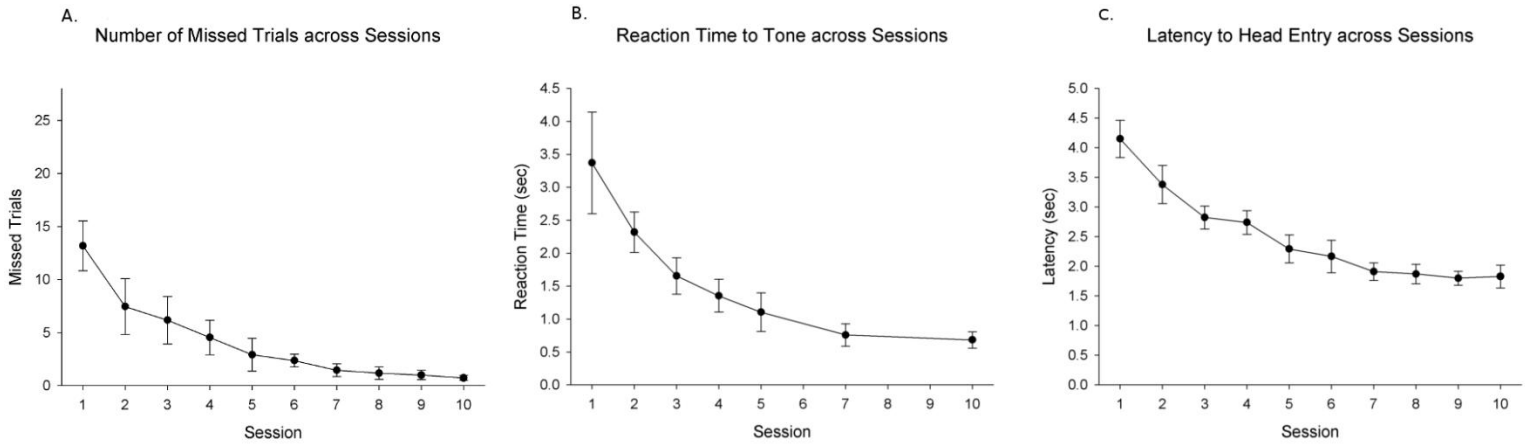


Figure 3: Groups of animals acquired the cue-respond reward relationship over 10 seesions of conditioning. A. Number of missed. B. Reaction time to tone (operationally defined as the time difference between tone onset and the next time-in approach). C. Latency to head entry (operationally defined as the time difference from tone onset to the next time-in head entry). All mesurements was averaged across all animals for individual sessions. Error bar represents standard error of the mean

Fig. 4

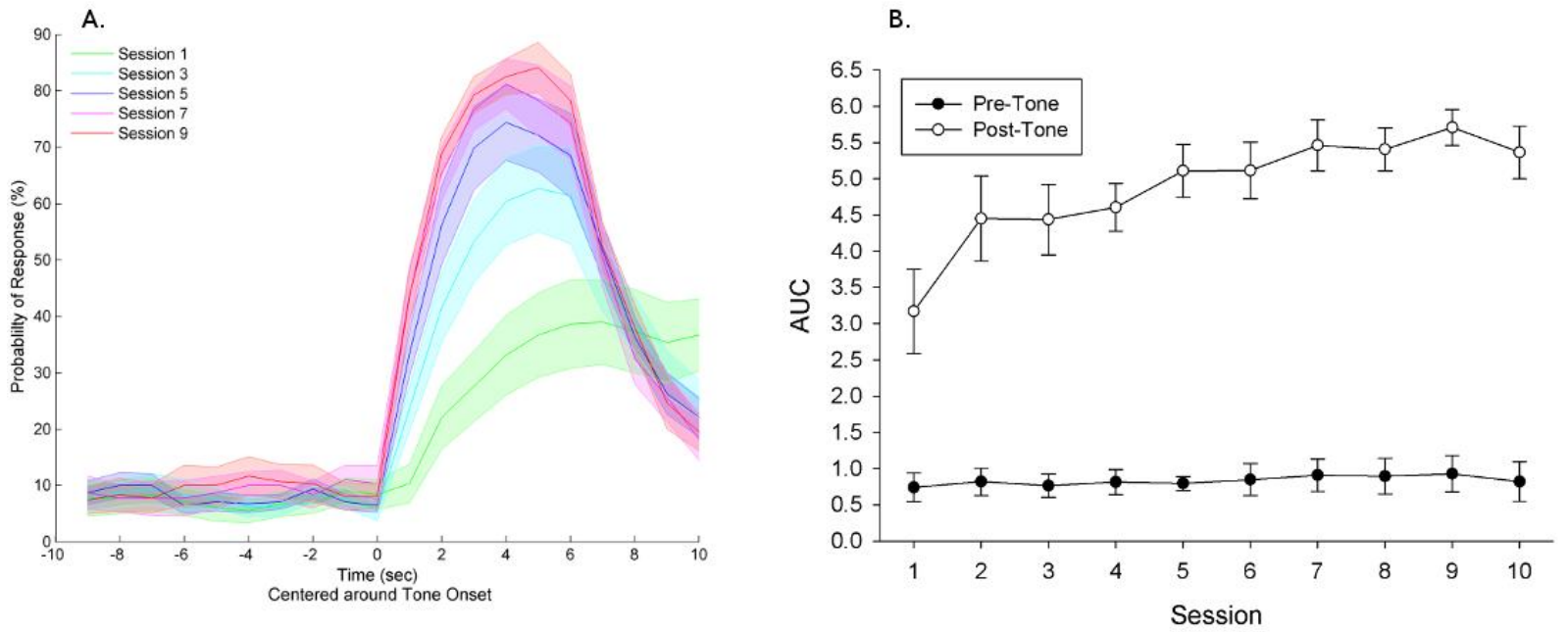


Figure 4: Probability of response around tone onset. A. Probability of response around tone onset. Results from every other session were shown for visualization. Solid lines represent probability of response; shaded bands around the solid line represent standard error of the mean. The probability of response after the tone is higher after the tone onset and increase over sessions. B. The area under the probability to response curve (AUC) pre- vs. post tone onset over sessions.

Fig. 5

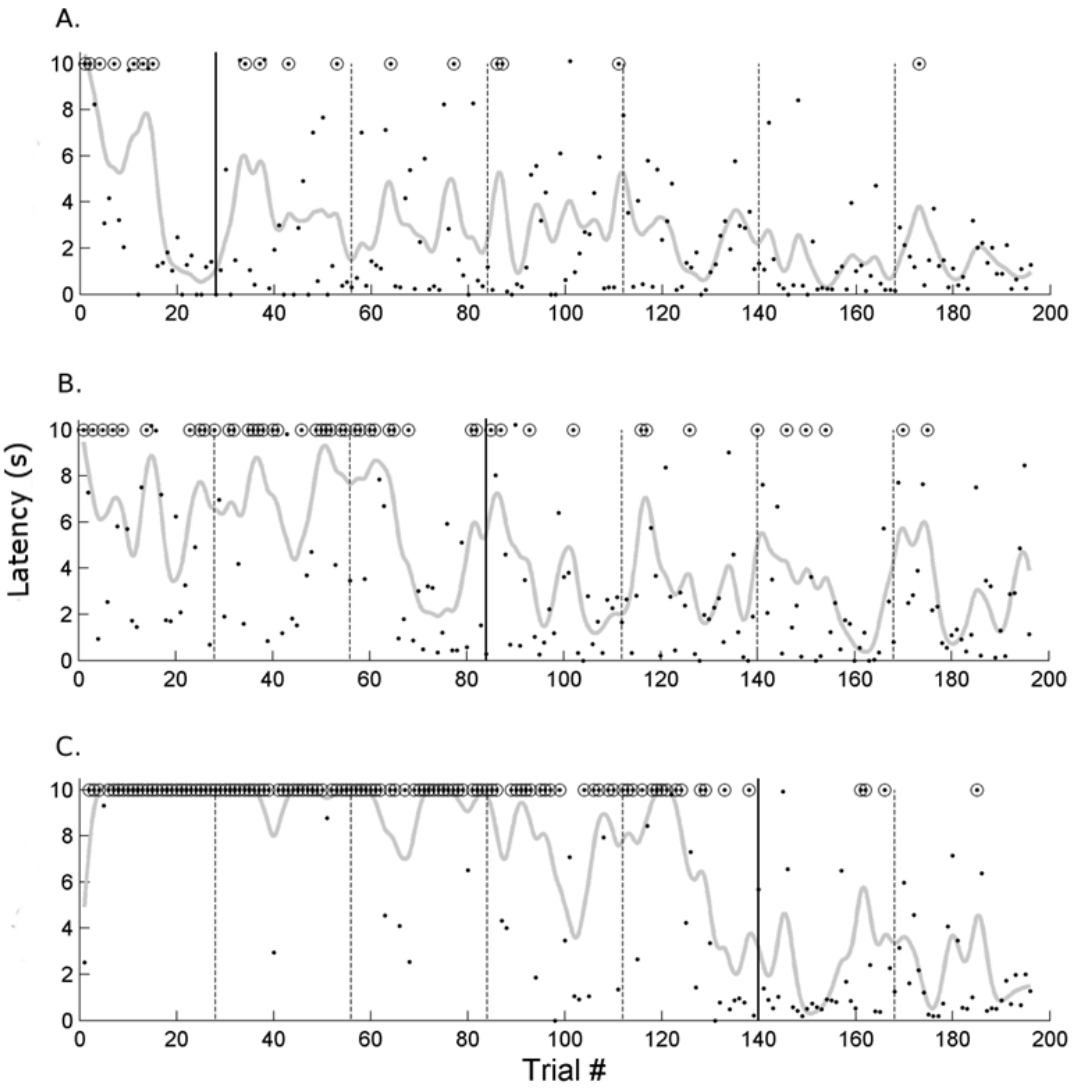


Figure 5: Response latency throughout conditioning trials for representative animals. Response latency to the tone was plotted for every trial (labeled consecutively) for sessions 1, 2, 3, 4, 5, 7 and 10. Black dots represent response latency for individual trials. For trials that animal fails to initiate a response, latencies were plotted as 10 seconds (longest possible latency). An open circle surrounding a black dot was used to indicate such trials. The grey line shows a spline curve interpolation of the latencies. Dashed vertical lines separate different sessions. Sessions before the black vertical line are pre-inflection point sessions, whereas sessions after the black vertical lines are post-inflection point sessions. A. Change in response latency across trials for a fast learner. B. Change in response latency across trials for an intermediate learner. C. Change in response latency across trials for a slow learner.

Fig. 6

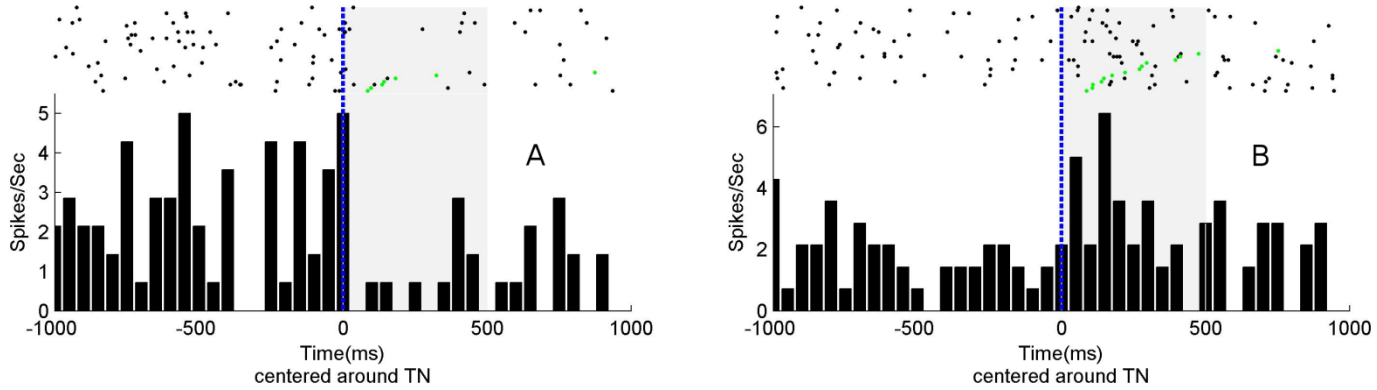


Figure 6: Example neural activity during tone presentation. The two figures were constructed around tone onset. Each figure consists of one raster plot (top) and one PETH (bottom). Each row in the raster plot represents one trial. Trials were centered around tone onset, with the blue dashed line indicating the tone onset. Black dots represent action potentials. Green dots in represent the initiation of cued approach. The area shaded in grey indicates the tone presentation. The PETH displays the average number of action potentials in a given 50 ms bin. Neuron A displays a decrease during tone presentation, whereas Neuron B displays an increase in activity during tone presentation.

Fig.7

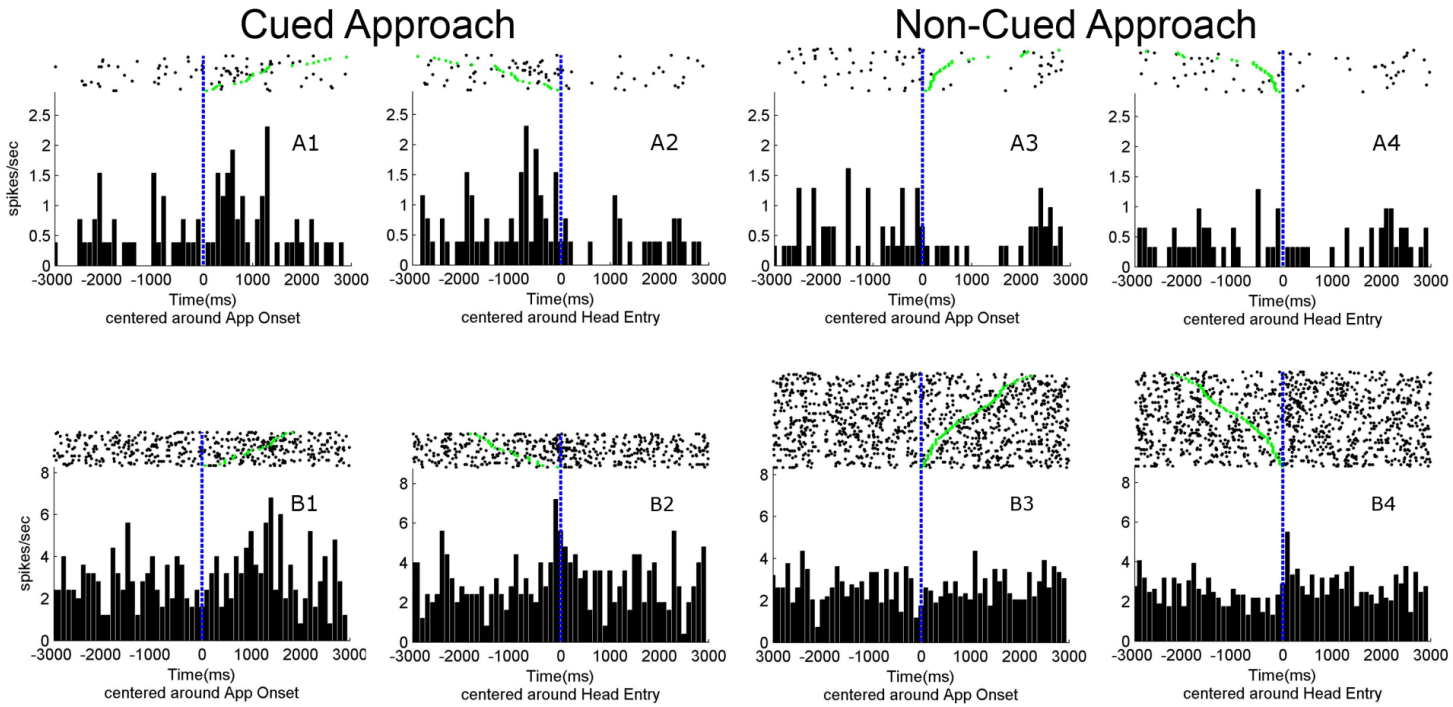


Figure 7: Example neural activity during cued approach and non-cued approach. Panels in the top row (A1, A2, A3, A4) were constructed from the neuronal activity of one neuron. Panels in the bottom row (B1, B2, B3, B4) were constructed from the neuronal activity of another neuron. Panels in the same column were constructed around the same behavioral event. The four columns were constructed around cue approach onset, cued approach end (i.e. head entry), non-cued approach onset, and non-cue approach end (i.e. head entry) respectively. Each panel consists of one raster plot (top) and one PETH (bottom). Each row in the raster plot represents one trial. Trials were centered around a given behavioral event, with the blue dashed line indicating the behavioral event. Black dots represent action potentials. Green dots in columns 1 and 3 represent the end of approach (i.e. head entry). Green dots in column 2 and 4 represent the onset of approach. Trials were sorted according to the duration of approach. Between the blue dashed line and the green dots is the approach behavior. The PETH displays the

average number of action potentials in a given 100 ms bin. Both neurons display increased firing rate during cued approach and decreased firing rate during non-cued approach in the above example.

Fig. 8

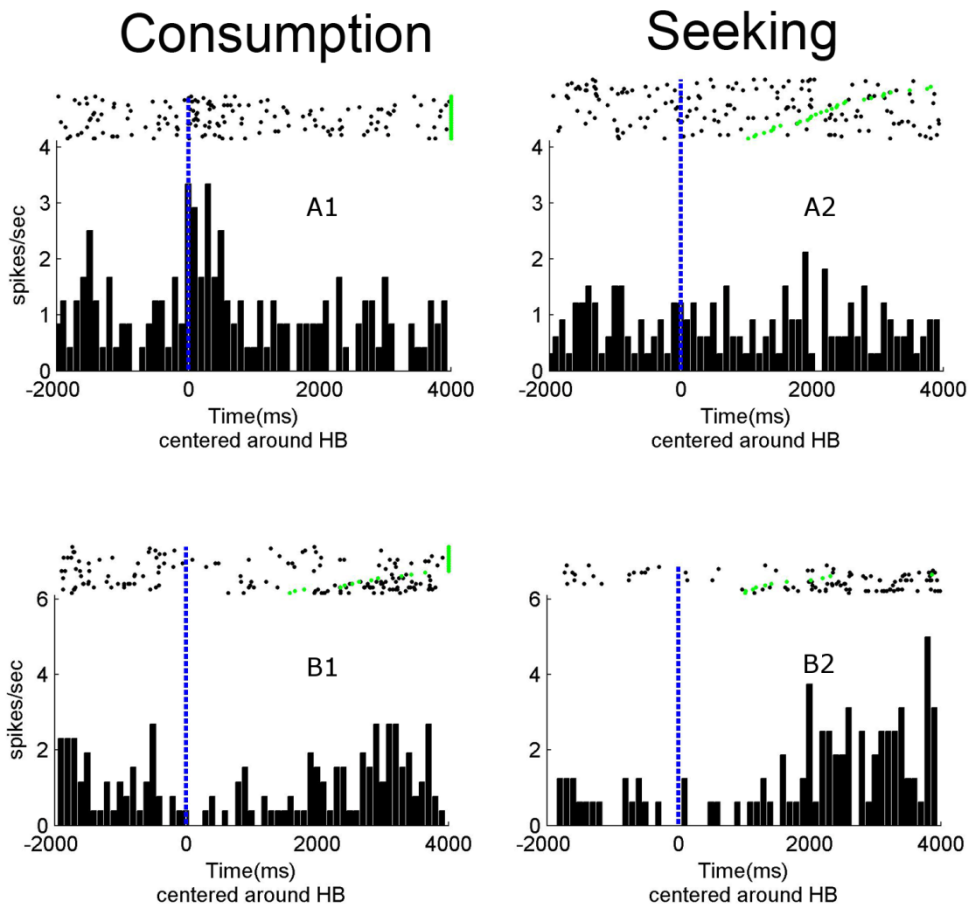


Figure 8: Example neural activity during consumption and seeking. Panels in each row were constructed from the activity of one neuron. Panels in the same column were constructed around the same behavior event. The two columns were constructed around onset of consumption and onset of seeking respectively. Each panel consists of one raster plot (top) and one PETH (bottom). Each row in the raster plot represents one trial. Trials were centered around consumption/seeking, with the blue dashed line indicating the behavioral event. Black dots represent action potentials. Green dots in column 1 represent the end of consumption. Green dots in column 2 represent the end of seeking. Trials were sorted according to the duration of consumption/seeking. The PETH displays the average number of action potential in a given 200 ms bin. The neuron shown in the top row displayed an increase in firing rate early during consumption but modulation of firing was not obvious during seeking. The neuron shown in the bottom row displayed decreases both during consumption and seeking.

Fig. 9

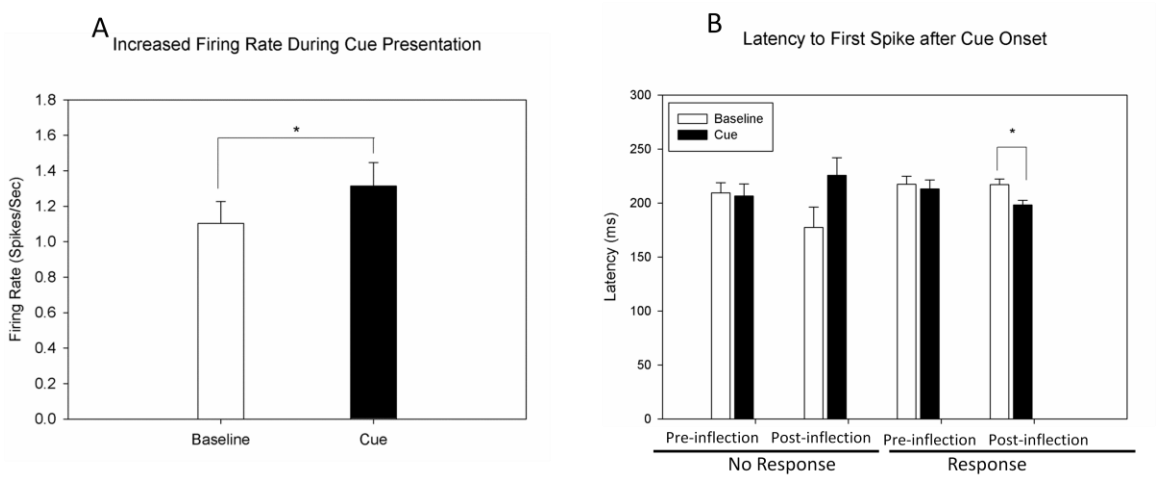


Figure 9: Neural activity during cue presentation. A. An increase in firing rate was observed during cue presentation in comparison to baseline. B. A reduction in neural response latency was observed during cue presentation in comparison to the baseline post-inflection point in the trials where the animals will initiate a behavioral response. The following number of neuron-trials were utilized in the analysis:1078 pre-inflection point trials where the animal did not make a behavioral response, 243 post inflection trials where animal did not make behavioral responses, 1707 pre-inflection trials where animals made a behavioral response, and 4980 post-inflection trials where the animals did make a behavioral response.

Fig.10

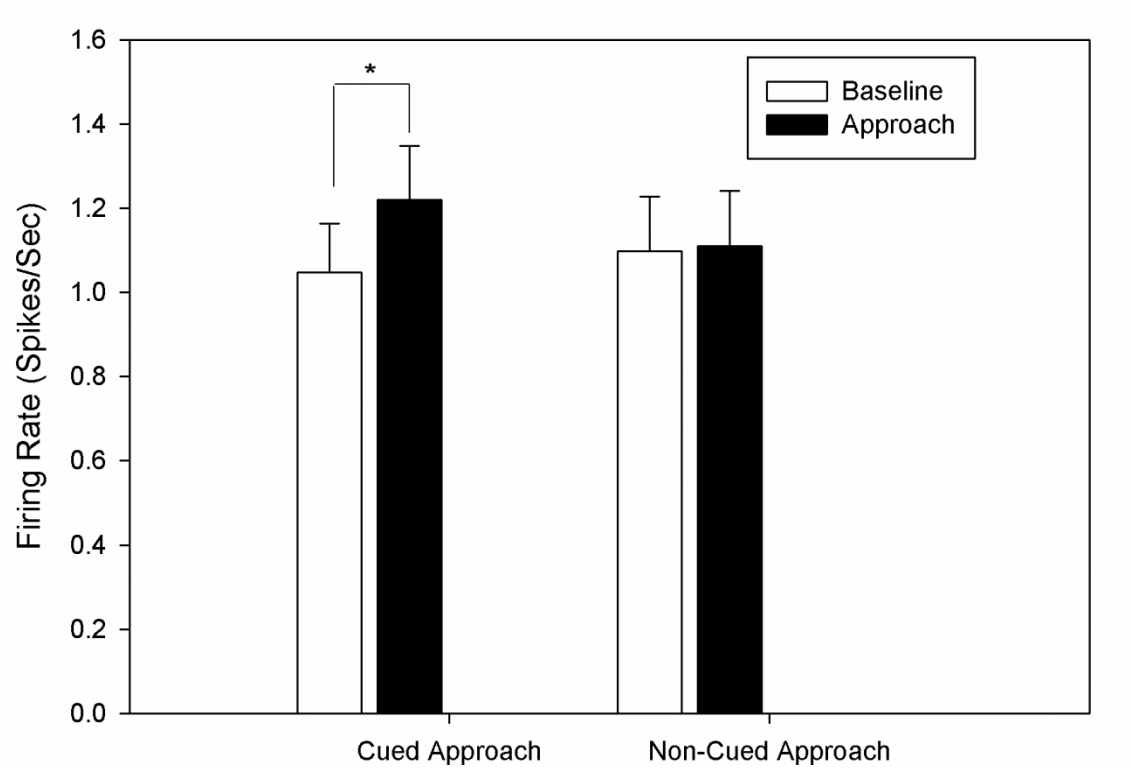


Figure 10: Neural activity during approach (see text for details).

Fig. 11

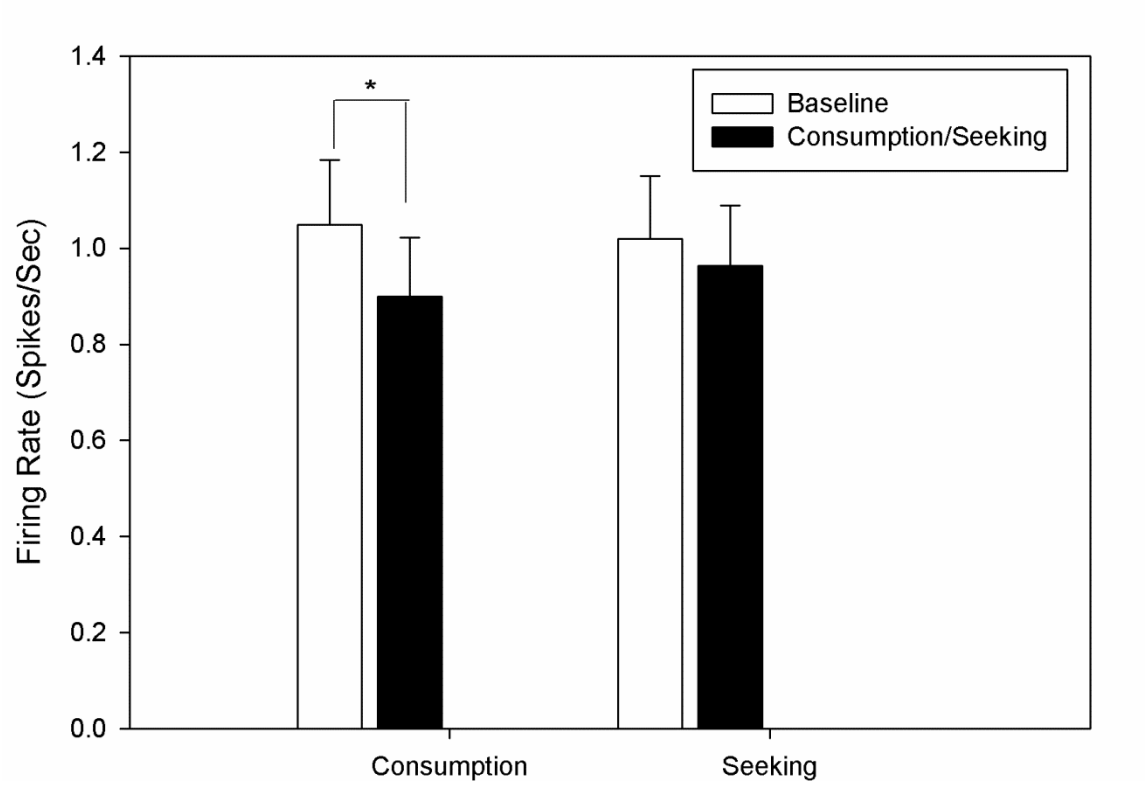


Figure 11: Neural activity during reward consumption and reward seeking (see text for details).

Fig. 12

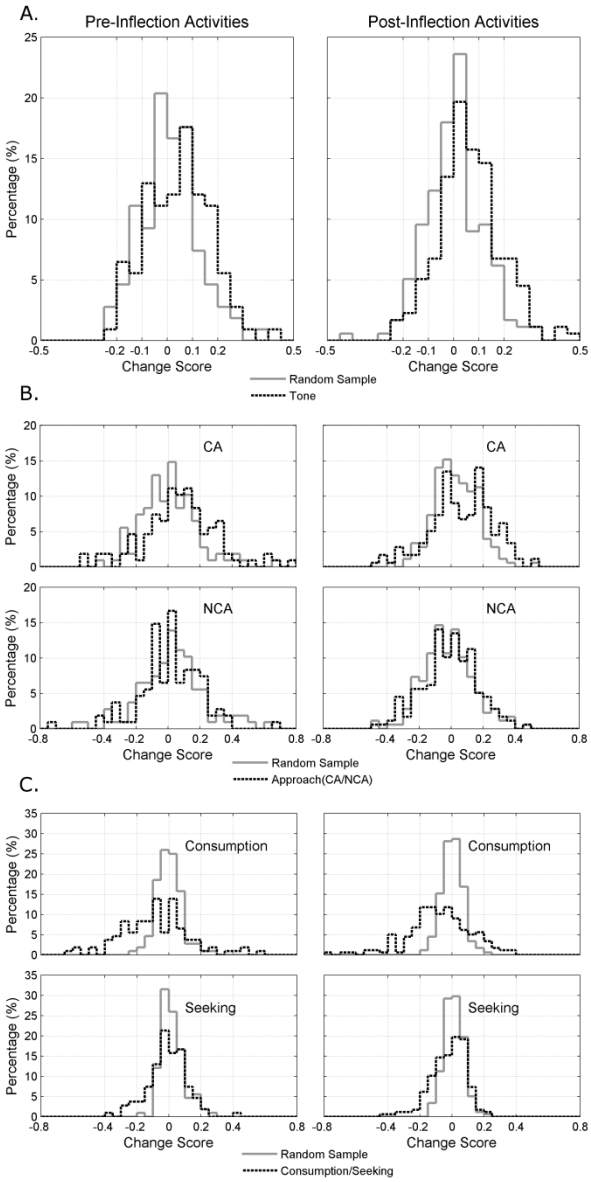


Figure 12: Distribution of change score during different reward-related events. Distribution of change score during different reward-related events (black dashed line) was plotted against the distribution of change score for the firing rate during a randomly sampled time period with matching duration to the reward related event. A. Distribution of change score during tone presentation. B. Change score during approach behavior. C. Change score during consumption and seeking.

Fig. 13

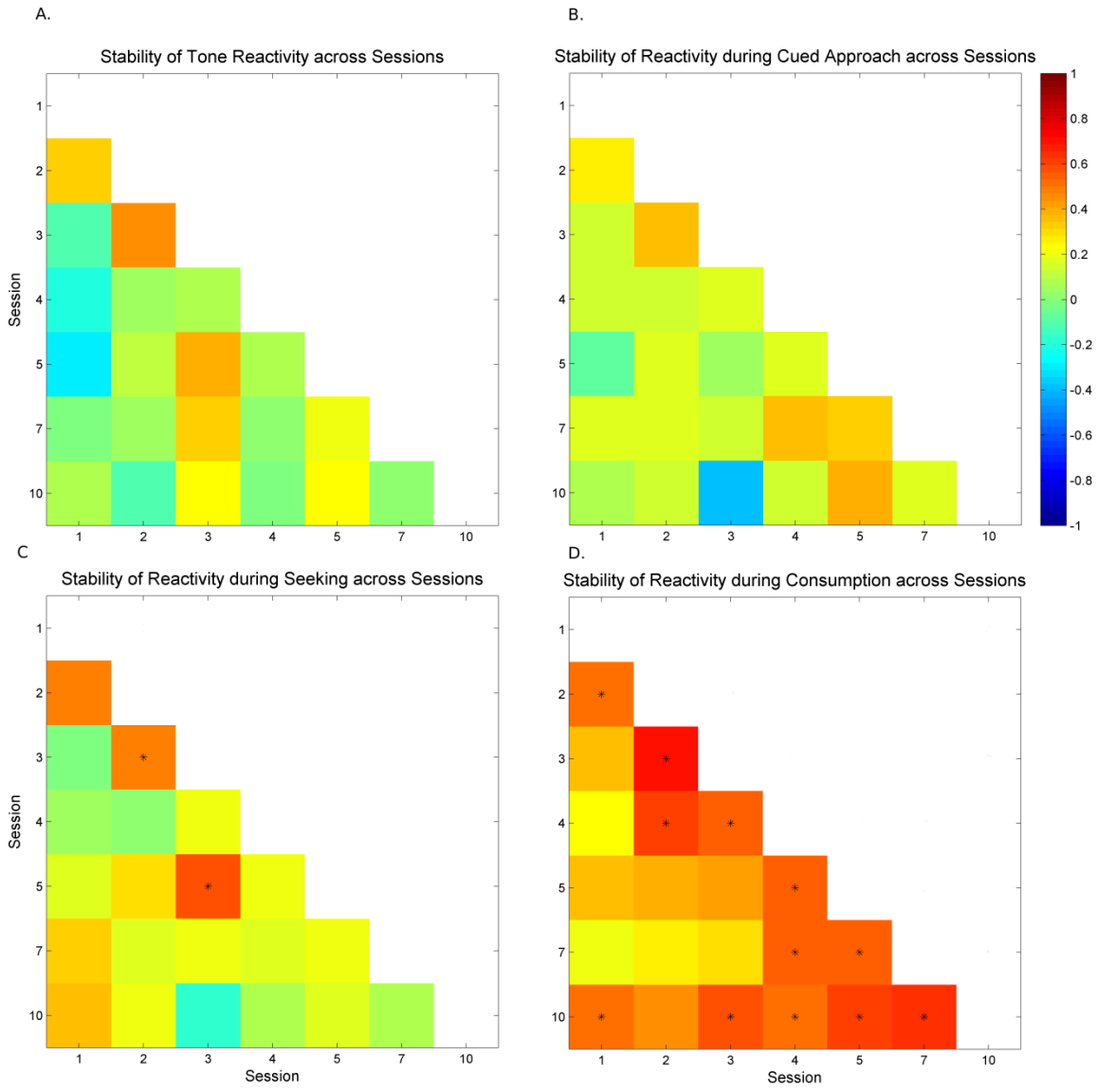


Figure 13: Stability of neural activity across session. Correlation for neural activity was computed between pairs of sessions. The correlation coefficients were visualized in heat maps. Warmer color indicates stronger positive correlation, i.e. higher stability, whereas cooler color indicates weaker positive correlation, i.e. lower stability. Significant correlation was labeled with "*". The stability of neural activity during tone presentation (A), cued approach (B), and seeking (C) is low, whereas the stability during reward consumption (D) is high.