LONGITUDINAL ANALYSIS OF CHANGES IN NUCLEUS ACCUMBENS REWARD PROCESSING ACROSS PROTRACTED COCAINE SELF-ADMINISTRATION

By

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ABSTRACT OF DISSERTATION

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Theories suggest that drug dependence develops as drug use transitions from acute ingestion to protracted drug-taking behavior. Studies in both humans and primates have shown that striatal circuitry is intimately involved in these long term changes. Notably, the ventromedial portion of the striatum (i.e., the nucleus accumbens) has a long established role in supporting the reinforcing properties of abused drugs and in modulating drug-seeking behaviors. To date, however, a thorough longitudinal analysis of the functional changes in accumbens activity during the transition from acute to chronic drug use has yet to be conducted. Thus, the goal of the present experiment was to track single neuron activity across 24 days of chronic, long-access (6 hours/day) self-administration training and examine changes in the tonic firing patterns of accumbens neurons as well as changes in firing patterns during on-drug locomotion, approach to the operant, and operant responding. Finally, the relationship between drug level and firing rate was examined longitudinally. To accomplish this, animals were shaped and

subsequently trained to self-administer cocaine by performing an operant head movement on a variable-interval schedule. Single-units were recorded across self-administration sessions using an array targeting the nucleus accumbens core and shell. Data recorded from wires that had been localized to the accumbens core, shell, or core/shell border using antibodies raised against Calbindin D28-K were then analyzed longitudinally using a generalized linear model. Results from the present study establish that continued cocaine use corresponds to stable negative correlations between drug level and the firing rates of accumbens neurons. Moreover, dynamic changes in the firing patterns of accumbens neurons during operant responding were observed over sessions. In contrast, firing during locomotor behaviors and goal-directed approaches was not different from baseline firing rates. Overall these results suggest that the accumbens plays a specific role in response-reinforcement learning and may exhibit plastic changes in firing patterns as learning occurs. Moreover, the strength of learned associations may be influenced by the effects of cocaine, which produces a robust effect on accumbens neurons, even upon first exposure.

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1. Introduction:

1.1—Cocaine Addiction:

Drugs are used illicitly by an estimated 22.6 million Americans each year (8.9% of the population). Amongst the myriad of abused illicit drugs, cocaine is one of the most highly abused, with an approximate 0.6 million new users each year. Cocaine use tends to be highest among young adults, ages 18-25, with approximately 1.5% of the population reporting use of the drug. On the other hand, the prevalence of cocaine use is slightly lower in adolescents (ages 12-17) or older adults (ages 26+), with approximately 0.5% of the population within each of these age groups reporting illicit cocaine use. Accordingly, the average age at which an individual initiates cocaine use is 21.2 years of age, and 71.6% of cocaine initiates are over the age of 18. Moreover, cocaine use also tends to be higher among males (0.8%) than in females (0.4%; SAMHSA, 2010).

In 2010, more than 7.1 million individuals (0.4% of the population) met the criteria for drug abuse or dependence. Of the 1.5 million self-reported cocaine users in the United States, more than 1 million individuals meet the criteria for substance abuse or dependence (a decline from 1.5 million in 2002; SAMHSA, 2010). Drug dependence occurs when a drug is used repeatedly for non-medical purposes in such a manner that a strong psychological or physiological dependence develops. The key characteristics of dependence are (1) perseverative drug seeking despite health problems, legal problems, or interference with personal obligations (2) escalation of drug intake despite attempts to control drug use, and (3) negative physical or psychological states that coincide with the cessation of drug use. These symptoms are thought to be brought about by changes in the brain that occur following protracted drug abuse. This drug-induced plasticity is also

believed to be involved in drug relapse—a chronic problem for substance-dependent individuals (American Psychiatric Association 1994; Koob 2008, 2009, 2009b; Koob & LeMoal 2008). Importantly, 699,000 individuals seek treatment for cocaine addiction each year, making research into its causes and treatment a primary goal of the National Institutes of Health.

1.2—Statement of Problem and Project Objective

The nucleus accumbens (NAcc), a component of the limbic system, has long been implicated in processing drug-seeking behaviors (Roberts et al., 1977). Furthermore, studies have shown that changes in tonic activity occur in the accumbens when comparing its activity following acute and chronic cocaine administration (Macey et al., 2004; Porrino et al., 2008). Given these data, along with evidence showing corresponding changes in the dorsal striatum (Porrino et al., 2008), theories of addiction have suggested that changes in striatal processing are involved in the development of substance dependence as well as 'habitual' or 'automatic' drug-seeking behaviors. Specifically, these theories suggest that the accumbens is involved early in training when behaviors remain goal-directed but that control over drug-seeking behaviors shifts to the dorsal striatum as drug-seeking behaviors become habitual (Everitt & Robbins, 2005; Tiffany, 1990).

Despite evidence showing shifts in tonic measures of striatal activity, evidence for changes in moment-to-moment signaling of striatal neurons during drug-seeking behaviors is necessary to support or refute predictions made by the aforementioned theories. Specifically, do these tonic changes actually reflect changes in the action

potential activity of neurons in the accumbens? Moreover, these changes must be thoroughly tracked over time in order to effectively test whether neural activity systematically shifts, as hypothesized, in this model of the transition from acute drug use to substance-dependence. With this in mind, the goal of the present study was to track changes in the firing patterns of single neurons in the nucleus accumbens over the course of protracted drug use in order to determine if firing patterns related to goal-directed behaviors are a) present early in training and b) are absent late in training following chronic drug administration. Evidence for both of these conditions would provide support for models of habitual processing while the absence of either condition would oppose these predictions. Our objective was to test the hypothesis by taking advantage of the high spatial and temporal resolution of recording single unit activity and yet by analyzing activity of the whole sample, rather than focusing only on isolated instances of phasic firing patterns that could be outliers or that could be undetectable by the tonic approaches mentioned above.

1.3—Functional Limbic connectivity:

It has been proposed that the limbic system represents a final common pathway for the reinforcing effects of all drugs of abuse. While this may over-simplify the limbic system's role in the reinforcing effects of drugs of abuse, evidence does support the notion that the mesolimbic system is involved in some aspect of substance dependence for nearly every abused drug. Thus, understanding limbic structures and their function is an important first step towards deciphering the effects of abused drugs on the brain.

Structures typically considered to be part of the mesolimbic dopamine system include the Ventral Tegmental Area (VTA), the Nucleus Accumbens (NAcc), the

Amygdala, the Hippocampus (HPc), and sometimes the Bed Nucleus of the Stria Terminalis (BNST). These structures are highly influenced by cortical activity in the Prefrontal Cortex (PFC) and Cingulate cortex. Mesolimbic structures are also thought to influence behavior via connections to Basal Ganglia structures, such as the Ventral Pallidum (VP), which subsequently influence brainstem motor pattern generators and thalamic structures such as the Mediodorsal Thalamic nucleus (MDT; Smith et al., 2009). Thus, it has been proposed that limbic circuitry is involved in translating hedonic or motivational signals to behavioral output (Mogenson et al., 1980).

Limbic structures are responsible for processing both rewarding and aversive stimuli. Furthermore, evidence has suggested that both associative and instrumental conditioning are processed within the limbic system, but that each may involve separate structures. It has been shown that associative information is primarily processed in the amygdala. Contingent but response *independent* associations have been shown to depend on the basolateral amygdala (Maren 2003), while the central nucleus of the amygdala is necessary for stereotyped responses to learned associations (e.g. conditioned approach; Baxter & Murray, 2002). It is thought that associative information from the basolateral amygdala influences behavior via a substantial projection to the orbitofrontal cortex (OFC), which in turn projects to pre-motor and motor cortices. Based on available evidence, theories of associative learning predict that the connection from the BLA to OFC is necessary for attributing value to a stimulus, selecting an appropriate action based on stimulus valence, and (when necessary) updating the value of a stimulus (Schoenbaum et al., 2003).

Instrumental behaviors are influenced by signals from the amygdala (Stuber et al. 2011), but they do not require its involvement (Maren, 2003). Instead, substantial evidence has implicated the NAcc in processing instrumental behavior (Carelli and Deadwyler, 1993, 1994; Ghitza et al, 2004; 2006). Moreover, it has been shown that the NAcc oppositely processes appetitive and aversive outcomes. Specifically, dopaminergic concentrations in the NAcc are increased following the presentation of appetitive stimuli, but are reduced following the presentation of aversive stimuli (Roitman et al, 2008; Wheeler et al, 2011). Accordingly, these changes in dopaminergic concentration correspond with decreases (appetitive) or increases (aversive) in the firing rates of medium spiny neurons (Roitman et al., 2005).

Neural information processed in the NAcc seems to represent the amalgamation of input from multiple sources. As mentioned previously, appetitive and aversive stimuli are accompanied by changes in dopaminergic input from the VTA. Moreover, signaling from the lateral hypothalamus has widespread influence on accumbens firing via direct opioidergic connections to cholinergic interneurons in the NAcc (Rada & Hoebel, 2001; Hoebel et al, 2007). Finally, the accumbens is also influenced by glutamatergic projections from the Amygdala (Stuber et al. 2010), PFC (Sesack et al., 1989), and hippocampus (Valenti et al. 2011). Overall, this positions the NAcc to incorporate information about context, learned cues, autonomic function, and motivational state or—perhaps more importantly—to integrate information from nearly every structure in the mesolimbic system.

Subsequently, the NAcc—which comprises a core and shell—is able to affect motor behavior via topographic projections from core and shell to their primary output

structure—the ventral pallidum (VP). The VP also integrates projections from the VTA and basolateral amygdala (Heimer and Wilson, 1975; Nauta et al. 1978; Heimer, 1978; Haber and Nauta 1983; Zahm and Heimer, 1990; Fuller et al. 1987; Sesack et al. 1989; Klitenick et al. 1992) and has been implicated in the goal-directed behaviors involved in drug-seeking (Root et al., 2013). Thus, firing patterns in the NAcc are likely translated into patterned motor behavior as limbic signaling transitions from the striatal system to the pallidal system and on to thalamocortical and mesencephalic locomotor systems.

1.4—Neural Substrates of Cocaine Addiction:

The longitudinal physical and psychological effects of cocaine are particularly dynamic because of the plethora of neurotransmitter systems affected by the drug. In particular, cocaine's modulation of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) transmission produces an ever changing sequence of positive and negative symptoms. Positive effects range from alertness and increased energy to euphoria while the negative effects include hypertension, anxiety, and paranoia (Schank et al, 2008). The juxtaposition between the positive and negative effects of cocaine is attributable to the non-selective neurochemical effects of the drug. Indeed, cocaine is often considered a "dirty drug" because it up-regulates extracellular concentrations of the three major monoamines—NE, DA and 5-HT—via effects on their respective transporters (DAT, NET & SERT; Reith et al, 1997). Among these, cocaine's affinity is thought to be highest for 5-HT and DA (Cameron & Williams, 1994) although its effects on NE are still substantial (Han & Gu, 2006; Reith et al., 1997). Appropriately, many of these

monoamines co-exist in nuclei that are implicated in cocaine's reinforcing effects (e.g., the nucleus accumbens and ventral tegmental area; Reith et al, 1997).

Empirical evidence has suggested that the reinforcing effect of cocaine can be attributed mostly to DA; however, 5-HT and NE are thought to act in synergy with dopamine in order to modify its effects. Appropriately, DAT reuptake inhibitors are readily self-administered, but NET and SERT reuptake inhibitors are not. Moreover, only DA neurons support intracranial self stimulation (Filip, 2005). Dopamine has also been implicated in cocaine induced hyperlocomotion (Uhl et al, 2002) and the development of stereotyped movements (Kuczenski et al., 1991; Aliane et al., 2011).

Norepinephrine plays an important role in many of the cardiovascular/ sympathomimetic effects of cocaine (Sofuoglu et al, 2009). Norepinephrine acts on muscles and tissue peripherally to produce changes in heart rate etc., but also acts within the BNST and CeA to influence autonomic functions centrally via the hypothalamus. Changes in noradrenergic transmission following cocaine abuse seem to cause lasting changes in the brain. In rhesus monkeys, it has been shown that NET density is increased in the BNST following repeated cocaine exposure (Weinshenker et al, 2007). It is likely these changes in NET density and the accompanying dysregulation of noradrenergic signaling produce many adverse effects of cocaine following the cessation of drug use (Macey et al, 2003). In fact, it has been shown that cocaine-induced anxiety is abolished in mice lacking dopamine-β-hydroxylase (an enzyme that converts DA to NE), but is reinstated following a restoration of NE (Schank et al., 2008). Importantly, the BNST is positioned to reduce excitation, and increase inhibition of the VTA. Thus, the noradrenergic system and dopaminergic system may work in opposition to one another.

Noradrenergic dysregulation may also explain the symptoms of psychological withdrawal observed following the termination of cocaine use. Administering the alpha-2 antagonist yohimbine (which prevents autoreceptor activation, thereby preventing the NE system from shutting down) during acute withdrawal was shown to increase panic and anxiety (Macey et al, 2003). Clonidine (an α2 agonist), on the other hand, attenuates these symptoms (Smith and Aston-Jones, 2008). Lastly, the BNST shows increased FOS during withdrawal and Corticotropin-releasing factor antagonists can reduce withdrawal-induced anxiety (CRF is a downstream product of NE activation in the BNST; Smith & Aston-Jones, 2008).

The most concrete role for NE in drug addiction seems to be its role in stress-induced reinstatement. Noradrenergic projections from the lateral tegmental area to the CeA, BNST, and septum seem critical for these effects (Leri et al, 2002). Sub-regionally, α2 agonists have been shown to attenuate stress induced reinstatement. Similarly, β1/β2 antagonists injected into the BNST and CeA cause a dose dependent reduction in stress induced reinstatement. As stated above, and confirmed by reinstatement studies, the importance of the noradrenergic innervations of the BNST and CeA in stress-induced reinstatement likely involves the interaction of NE with the CRF system (Leri et al, 2002; Weinshenker et al 2007; Macey et al, 2003).

The role of 5-HT in cocaine addiction is perhaps the most complex given the surfeit of 5-HT receptor types (7) and receptor subclasses (16 known; Filip, 2005). One comprehensive theory for the function of 5-HT in addiction is that it may act to set the "gain" for NE and DA. Given the aforementioned roles of NE and DA, this would suggest that 5-HT plays a role in both the positive and negative effects of the drug. For

example, serotonin reuptake blockers are rewarding in dopamine knockout mice, but can enhance the aversive effects of cocaine in combination with NET inhibition (Filip et al, 2005). Still, empirical evidence for the interaction of 5-HT and DA seems far more prevalent than evidence for the 5-HT/NE interaction.

Serotonin and dopamine heavily interact in the VTA and NAcc (Mateo et al, 2004). The VTA is innervated by the dorsal raphe nuclei and 5-HT acts pre-synaptically on GABA terminals in order to inhibit GABA (thereby disinhibiting DA neurons). In fact, cocaine has been shown to decrease GABA-B mediated IPSPs in a dose dependent manner (Cameron & Williams, 1994). Moreover, depleting serotonin in vitro abolished the observed change in GABAergic IPSPs following cocaine application. Thus, it seems that 5-HT is necessary for overcoming GABAergic inhibition on dopamine neurons and allowing cocaine to have its full effect. Indeed, many have suggested that, in humans, cocaine's affinity for DA is not high enough to produce the observed pharmacological and behavioral effects at behaviorally relevant levels, if considering DA alone.

Given the robust changes in neurochemical signaling induced by cocaine and other drugs of abuse, it is no surprise that substantial changes in functional connectivity occur following prolonged drug use. For example, it is known that neurochemical changes cause drug induced plasticity including changes in receptor density (Nader et al., 2002) and receptor sensitivity (Henry & White, 1991). Undoubtedly, these changes also correspond to changes in cellular physiology and lead to changes in reward processing (Waselus et al., 2013).

1.5—The Nucleus Accumbens and Drug Addiction:

One of the most important hallmarks of addiction is addicts' perseverative drug seeking. Drug-seeking behaviors often persist despite the overwhelming array of negative consequences associated with continued drug use (Tiffany, 1990; American Psychiatric Association 1994). Therefore, understanding changes in neural activity as drug abuse transitions from acute to chronic is pivotal for understanding the perseverative drug seeking behavior that becomes entrained in chronic drug users.

Theories of addiction have suggested that long-term changes in striatal activity may be responsible for the transition from goal-directed drug seeking behaviors to automatic or habitual responding (Everitt and Robbins, 2005; Fuchs et al, 2006; Tiffany, 1990). Goal-directed behaviors are those whose responding is outcome driven, while habitual responding develops over repeated stimulus-response associations such that responding becomes stimulus elicited (i.e., insensitive to changes in the outcome; Dickinson, 1985). This transition from a goal-directed behavior to an S-R habit is thought to involve neuroadaptations in striatal dopamine transmission (Di Chiara, 2002), glutamate transmission (Thomas et al, 2001; Martin et al, 2006), or changes in executive control over striatal activity and behavior (Martin et al, 2006; Grace et al., 2007).

Evidence supporting these theories includes the data demonstrating that neural activity gradually shifts from the ventromedial striatum (i.e. nucleus accumbens) to the dorsolateral striatum across chronic drug SA (Letchworth et al, 2001; Macey et al, 2004; Porrino et al, 2004). Furthermore, changes in glucose utilization (Porrino et al., 2004), dopamine transporter (DAT) expression (Letchworth et al., 2001), D2 dopamine receptor expression (Nader et al., 2002) and the loss of dorsolateral striatum somatosensory specificity (Hanlon et al, 2009) have been observed across protracted drug use.

In the NAcc, previous work has shown that neurons exhibit discharges around the instrumental response after extended training (Carelli and Deadwyler, 1993, 1994; Ghitza et al, 2004; 2006). Furthermore, the magnitude of drug-induced reductions in tonic firing patterns becomes more precipitous late in training (session 15) when compared to early in training (session 2; Peoples et al., 1999). These tonic decreases in firing rate are related to cocaine pharmacodynamics and are more prevalent in the shell than the core late in training (Fabbricatore et al., 2009). Appropriately, different neuroadaptations in the core versus the shell are hypothesized to drive the persistent drug-seeking that leads to addiction (DiChiara et al., 2002). Finally, NAcc firing patterns become sensitive to drug-associated cues after protracted training (Ghitza et al., 2003). Together, these results appear to be inconsistent with the hypothesized decline in NAcc activity with chronic cocaine use.

1.6—Expected Outcomes and Limitations

Current theories of addiction (Everitt and Robbins, 2005) suggest that the accumbens will be involved in conditioned approach and/or operant responding initially (e.g. Chang et al., 1994), but that event related firing patterns in the accumbens will be absent late in training as drug abuse becomes chronic. One might also expect changes in the relationship between firing rate and calculated drug level, as plastic changes in accumbens neurons have been observed following protracted drug use (Waselus et al., 2013).

The transition from acute to chronic drug use in trained laboratory animals involves multiple simultaneous changes. Specifically, the learning that occurs over sessions corresponds to changes in animals' motor patterns/behavior. Moreover, animals

are predicted to undergo changes in drug sensitivity (e.g., sensitization and/or tolerance). To control for these changes, 'matched sets' of equivalent behaviors will be tracked over sessions for each of the events of interest in the present study. The use of behavioral equivalence is important for comparing neural measures in awake behaving animals while eliminating sensorimotor differences as a possible alternative explanation of neural results (Porrino, 1993). Notably, this type of matching excludes from analysis some behaviors that are not observed repeatedly across training. However, attempts to analyze data without this type of sensorimotor matching results in ambiguity when interpreting obtained results, thus necessitating the matching approach.

Typical analyses of neural firing patterns during cocaine self-administration are conducted by recording during a single session or during a small sample of sessions(e.g., Peoples et al., 1999; Ghitza et al., 2004). These studies focused on neurons exhibiting salient changes in firing during a single sessions (i.e., 'responsive neurons'). While identifying responsive neurons is important for understanding types of events that neurons in a given region are sensitive to, the present study instead focuses on modeling population trends over sessions using a sample of neurons that have been collected and retained longitudinally.

The current population-based analysis was explicitly chosen to determine the types of longitudinal changes in accumbens neurons that correspond to preclinical, long-access models of substance dependence that are capable of producing automatic/habitual responding. This population analysis allows for high resolution tracking of individual neurons over time, but avoids explicitly focusing on ‘responsive neurons’ that may not represent the population trend (i.e., which are in the minority). Notably, this approach

allows us to specifically test predictions made by current theories of addiction which propose that accumbens neurons are no longer involved in processing drug-seeking behaviors once responding becomes automatic and substance dependence develops. Being the first of its kind, the present study sets the stage for future longitudinal drug abuse studies. Accordingly, many relevant questions remain which are not answered by the present approach and are discussed as possible future directions for other longitudinal studies.

2. Materials and Methods:

2.1—Subjects and Surgery

Male Long-Evans rats (n=21) from multiple litters (Charles River, Wilmington, MA) were singly housed on a 12h: 12h light: dark cycle with dawn at 1030 h. Prior to surgery, subjects were given sufficient food to maintain a pre-operative weight of 325-335 g. Once subjects reached a stable weight, they were anesthetized with sodium pentobarbital (50 mg/kg I.P.) and given 10 mg/kg of atropine methonitrate (I.P.) as well as a 0.25 mg I.M. dose of penicillin (300,000 U/ml) to maintain respiratory function and prevent infection. To block post-surgical pain sensitivity, animals were administered subcutaneous injections of Marcaine (0.25 ml bupivaccaine HCl; 0.25%, Abbott Laboratories, Chicago, IL) spread over 8 injection sites. Anesthesia was maintained throughout the remainder of the surgery using intermittent doses of ketamine hydrochloride (60 mg/kg I.P.) and sodium pentobarbital (5-10 mg/kg I.P.).

During surgery, animals were implanted with an indwelling catheter in the right jugular vein and a 2 x 8 microwire array (Microwire Technologies, Heightstown, NJ; Micro Probe Inc., Gaithersburg, MD) of quad Teflon-coated stainless steel microwires in

the right nucleus accumbens (DV: -6.6mm, AP: 0.5 to 3.0 mm anterior to bregma, ML: 0.6 to 1.8 mm lateral from bregma; Paxinos & Watson, 1997), both of which were anchored to the skull using dental cement and five jeweler's screws. The diameter of each uninsulated microwire tip of the array was 50 μm. The two columns of the array were separated by 750 μm and the eight rows were separated by 270 μm (center of microwire tip to center of microwire tip). A ground wire (0.25mm diameter) with 5 mm of exposed wire was implanted contralateral to the microwire array 5.5 mm ventral to the skull surface. An Omnetics connector strip (Omnetics Corp., Minneapolis, MN) served as the headstage connection and was positioned with a mediolateral orientation just anterior to the interaural line and secured with dental cement.

Following surgery, animals were housed in the self-administration chamber for the remainder of the experiment. All animals were allowed to recover from surgery for at least 7 days prior to the start of training (Figure 1). While living in this chamber, a computer-controlled syringe pump delivered a 200 μL infusion of heparinized-saline to subjects every twenty-five minutes around the clock in order to preserve catheter patency. On days in which animals self-administered cocaine, heparinized-saline infusions were delivered during the 18 hours in which the experimental contingencies were not in effect. All Protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH, 1985; Publication 865–23) and have been approved by the Institutional Animal Care and Use Committee, Rutgers University.

2.2—Experimental Apparatus:

All experiments were conducted in a custom Plexiglas operant chamber measuring 24 cm x 34.5 cm x 34.5cm and housed inside of a sound attenuating chamber (~76 cm^3). Animals were attached to an intravenous fluid delivery system consisting of a single speed syringe pump (Razel Scientific, St. Albans, VT) which connected to a fluid swivel (Instech Laboratories Inc., Plymouth Meeting, PA) via 122 cm of tygon tubing and allowed animals to move freely about the chamber. A 35.5 cm spring leash was connected to the bottom of the fluid swivel and extended down to the head of the animal through a 5 cm hole in the top of each chamber. Catheters were contained inside of the spring leash and continued through the spring leash and a steel cannula on animals' heads into the animals' right jugular vein.

The photocell operant used for responding (Figure 2; Root et al., 2011) consisted of a series of six infrared-emitting diodes capable of transistor-transistor logic (HOA-6299, Honeywell, Morristown, NJ), which were positioned along a 50º arc over 69 mm. Each photocell emitted a beam with a 5.59 mm diameter and an 880 nm wavelength. The photocell apparatus was attached to the back left corner of the operant chamber with the lowest photocell positioned ~13 mm from the floor of the operant chamber. From this point forward, the lowest photocell will be referred to as photocell one with incrementing photocells referred to as two through six. Unique hexadecimal codes were registered for each individual photocell break.

During shaping and training a 750 Hz tone (70 dB) was used to signal the completion of the operant response (below) using a custom tone generator (M.B. Turnkey Design, Hillsborough, NJ). All experimental apparatuses were controlled by a PC running MED-Associates hardware and software (St. Albans, VT). During hours when self-

administration sessions were not in effect, a Plexiglas block (50.8 mm x 50.8 mm x 152.4 mm) was inserted in the corner to block the photocell device and prevent extinction learning. Ventilation and masking noise for each chamber was provided by a series of shaded pole blowers (Grainger Industrial Supply, South Plainfield NJ).

2.3—Self-Administration Training

Self-administration sessions began at 10:00 AM daily (7 days/wk) immediately following the illumination of the houselight. Animals were shaped over the first 3-5 days of self-administration using a fixed-interval (FI) 10s schedule of cocaine availability. Responses following the 10s timeout period were reinforced with a 0.24 mg/0.2 ml infusion administered over 7.5s. During shaping, animals were required to make head movements of increasing difficulty starting with a single-photocell break at photocell two. The response requirement was incremented by one photocell after seven successful responses were performed at a given benchmark until subjects were successfully breaking photocells two through five in a single upward movement (termed the 'criterion response'; Figure 2; Figure 4D). For all criterion and shaping responses, it was required that the completion of the movement occurred within one second of the initial photocell break. All successful responses during shaping and training were signaled by a 750 Hz tone (0.5 s duration).

Animals were transitioned from shaping to self-administration training once they had reached and performed 10 or more rewarded criterion responses for two consecutive days. During training conditions, subjects were allowed to load-up on a FI 10 s schedule for the first nine infusions. Criterion responses during the loading period produced a 0.24

mg/0.2 ml cocaine infusion administered over 7.5 s. Following the load-up period animals were transitioned to a pseudo-random variable-interval (VI) 30 s schedule for maintenance responding. The first ten responses during the maintenance period produced a 0.12mg/0.1ml infusion over 3.75 seconds. Subsequently, animals were transitioned to their maintenance dose of 0.06mg/0.05 ml delivered over 1.875 seconds. For both load up and maintenance, if an animal failed to produce a response within 1 minute after the VI 30s had elapsed, the cocaine availability period ended and a new interval began. Training and shaping lasted a total of 24 sessions (Figure 1)

2.4—Drug level calculations

Assuming first-order pharmacokinetics, calculated whole-body drug levels were derived using the equation: $B_n = (B_{n-1} + D)\, e^{-KTn}$ (Yokel & Pickens, 1974);

Where:

T_n= the time since the previous cocaine infusion (min)

D= infusion dose/bodyweight (mg/kg)

B_{n-1}=cocaine level at time of last infusion (mg/kg)

K= rate constant of 0.028875, reflecting a 0.4-h metabolic half-life for cocaine (Nayak et al. 1976).

Drug level was calculated using the appropriate dose for each infusion (0.1775, 0.355, or 0.71 mg/kg/infusion). The rate constant for decay was selected based on a previous report by Nayak and colleagues (1976).

2.4—Single-Unit Recordings/Electrophysiological Procedures

Neural signals from all sixteen microwires were amplified at the level of the headstage using a harness with four quad-channel operational amplifiers (MB Turnkey Design, Hillsborough, NJ). Each harness connected to a fluid and electrical swivel (Plastics One Inc., Roanoke, VA**)** through which signals were fed to a preamplifier and filter (MB Turnkey Design, Hillsborough, NJ). The preamplifier differentially amplified (10x) the signal on each recording electrode against another wire on the implanted array that did not display a neuron. The filter then amplified (700x) and band-passed signals between 450 Hz and 10 kHz with a roll off of -1.5 dB per octave at 1 kHz and -6 dB per octave at 11 kHz. Finally, signals were digitized at a 50 kHz sampling frequency recorded using DataWave Technologies hardware and software (Longmont, CO). All signals were then stored for offline sorting and analysis.

Isolation of individual neural waveforms was conducted using spike sorting software from DataWave Technologies. Waveform separation was conducted by sorting neural discharges by a series of parameters for each waveform including peak voltage, spike height, valley voltage, time of peak voltage, voltage at user defined cursors, and the principal components derived from these parameters.

A matrix of scatter plots with all combinations of 2 of the above mentioned parameters was displayed in the sorting software. Each point within the scatter plots represented a parameter of a single recorded waveform. From these plots, oval or rectangular selection windows were used to separate individual neurons from background noise and other neural waveforms on the same recording channel. Waveforms were included for analysis if 1) Waveforms presented with canonical patterns of extracellular neural activity including a clearly defined action potential 2) the amplitude of putative

neurons exhibited at least a 2:1 signal: noise ratio 3) Parameters remained stable throughout the entire session and 4) an interspike interval (ISI) histogram showed that no discharges occurred during the neuron's natural refractory period (~2 ms). When multiple putative neurons occurred on a single recording channel, a cross-correlation between the two signals was used to confirm that the sampled spikes corresponded to distinct neurons.

Recordings of neural activity occurred approximately every other day over the course of self-administration training (Figure 1) with the goal of capturing changes in neural activity from early exposure through the escalation of drug intake that occurs across the first 2 weeks of training (Root et al., 2009, 2011; Barker et al., 2010). Given that the escalation of drug intake has been touted as the preclinical analog to human cocaine dependence (Ahmed and Koob, 1998), recordings taken during late sessions are taken to represent processing during chronic drug use. Thus, the schedule of recordings reflects processing during multiple facets of drug addiction.

2.5—Histological Procedures

Following self-administration training, animals were given an overdose of sodium pentobarbital (150-200mg/kg). Anodal current (50 μA for 3 sec) was passed through each microwire to produce a small lesion for localizing each microwire tip. Animals were then transcardially perfused with 0.9% phosphate buffered (PB) saline followed by either 10% phosphate buffered formalin (n=4; nissl staining procedure, described below) or 4% paraformaldehyde (n= 17; anti-calbindin D-28k immunohistochemistry procedure, described below). The brain was then removed and post-fixed in 4% paraformaldehyde (calbindin) or 10% PB formalin (nissl) before transitioning into a cryo-protectant solution

of 30% sucrose in PB. A microtome was used to make 50 µm thick coronal sections through the anteroposterior extent of the NAcc. Slices prepared with 4% paraformaldehyde were placed in 10 mL net wells filled with PB for immunohistochemistry (IHC), while those prepared with 10% phosphate buffered formalin were temporarily placed in wells with PB before slide mounting and staining with neutral red (nissl) and potassium ferrocyanide for microwire tips (Figure 1).

Immunohistochemistry was carried out under gentle agitation using a horizontal rotator (Laboratory-Line, Fisher, Pittsburgh, PA). Free-floating sections were washed for 3 x 10 m in PB, following which endogenous peroxidase was quenched by rinsing for 15 minutes in a 0.3% H_2O_2 solution. Sections were then washed in PB for 3 x 10m before treatment with blocking solution (4% bovine albumin + 0.3% triton X-100 in PB) for 1 h. Sections were then transferred into the primary antibody solution containing anti-calbindin d28k in blocking solution (Immunostar, Inc., Hudson WI) at a dilution of 1:5000 for a minimum of 16 hours at 4º C (gently agitated and refrigerated overnight). Following incubation in the primary antibody, sections were rinsed for 3 x 10m in PB and transferred to a solution containing Biotinylated secondary antibody against rabbit immunoglobulin (1:200 dilution; Vector Laboratories inc., Burlingame, CA, USA) in blocking solution for 1 h at room temperature. Again, sections were rinsed in PB (3 x 10m) prior to incubation with a solution containing Avidin-biotinylated horseradish peroxidase complex (ABC kit; Vector Laboratories Inc.) for 1 h. Finally, sections were rinsed in PB (3x 10m) before developing the peroxidase reaction with 0.05% 3, 3-diaminobenzide-4 HCl (DAB; Vector laboratories Inc.) and 0.003% H_20_2 for 2-3 m.

For both sections prepared with IHC and sections designated for nissl staining, individual slices were mounted onto gelatin-coated slides before incubation in a solution of 5% potassium ferrocyanide and 10% HCl to stain iron deposits left at the location of the lesioned microwire tip. Sections were then rinsed (3 x 1 m) in water to neutralize the reaction. At this point, slides designated for nissl staining were incubated in a 0.1% neutral red solution with 1% glacial acetic acid added to enhance nuclear staining. Nissl and IHC processed sections were then dehydrated through a graded series of EToH (70% 2m, 95% 2m, 100% 1m) before being transferred into a primary xylene solution for 2 m and then a second xylene solution while slides were cover slipped with Permount (Fisher, Pittsburgh, PA) and left to dry in a fume hood for 48 h.

Previous results from our laboratory (Root et al., 2013) and ongoing testing have shown that no staining occurs during this anti-calbindin-d28k IHC procedure when primary antibodies are omitted. The polyclonal anti-calbindin-d28k antibody was raised in rabbit against calbindin-d28k purified from bovine cerebellum and shows no cross-reactivity with other brain peptides, including calretinin, vasoactive intestinal polypeptide, somatostatin, substance P, and neuropeptide Y (Buchan and Baimbridge, 1988; Meek et al., 2008). Western blot analysis of the antibody detected a single band of 28 kD (Buchan and Baimbridge, 1988; Bell et al., 2005; Meek et al., 2008). According to the manufacturer and the scientific literature (Buchan and Baimbridge, 1988; Conde et al., 1994; Zahm et al., 1996; Bouilleret et al., 2000), specific immunostaining with the antibody was completely abolished by preadsorption with calbindind28k, which according to Buchan and Baimbridge (1988) was at a final concentration of 1 nM.

Calbdindin-D28k was used for IHC procedures because it has been shown to differentiate the NAcc core and shell (Fudge & Haber, 2002). Specifically, the NAcc shell is calbindin-d28k poor, while the core of the NAcc is rich with calbindin staining. A small transition zone (<100μm) exists between these structures with alternating strips of moderately calbindin positive and calbindin negative zones. Designation of microwire placement was made by a scorer blind to neural and behavioral results using a combination of atlas plates (Paxinos & Watson, 1997) and calbindin staining. In order to be considered within the accumbens core, lesions were required to be completely within the calbindin positive zone and within the dorsal and lateral bounds designated by Paxinos and Watson (1997). In order to be considered a shell neuron, lesions needed to be completely within the calbindin poor zone medial or ventral to the core, and also within the moderate-to-rich calbindin band which stains the medial and ventral edges of the shell (Figure 3).

2.6— Statistical Analyses of Behavioral data

Behaviors related to the head movement task were analyzed across sessions using a Generalized Linear Model (GLM) in SAS 9.4 (SAS Institute Inc.; Cary, NC, USA). The model included Session as a continuous variable. Behaviors analyzed using the GLM included: 1) Criterion responses (i.e. operant head movements), 2) Rewarded responses 3) Movement Distance, 4) Movement Duration, 5) Movement Velocity, 6) Drug consumption (total daily mg/kg), 7) Bodyweight. All behavioral data are reported as the mean ± the standard error of the mean (SEM). Alpha was set to 0.05.

2.6—Video Tracking

Animals' locations in the chambers were tracked using Datawave Video Bench software. A camera was mounted outside the chamber, approximately 23 cm from the front Plexiglas wall, and elevated 30 cm above the chamber's floor. In order to enable tracking, animals' recording harnesses were tagged with a pink tape which contrasted against a blue chamber background (Figure 4). Datawave Video Bench software was then used to track and record the X and Y coordinates of the pink marker every 0.033 s throughout neural recording sessions. The use of video tracking to extract specific, relevant behaviors is described below.

2.7 Neural Analyses:

The concept of behavioral equivalence (Porrino, 1993) is important for comparing neural measures in awake animals across conditions in order to eliminate sensorimotor differences as a possible alternative explanation of neural results. This is especially important in the present data, as the properties of observed behaviors can change across learning. Thus, behaviors tracked across longitudinal recordings were analyzed using 'matched sets' (Tang et al., 2007; 2009). Matched sets represent equivalent behaviors that are observed longitudinally. Specifically, similar behaviors were categorized based on parameters such as the movement distance or movement duration. When at least 5 movements were observed in a given category for four or more sessions, that behavior was included for longitudinal analysis.

Previous studies have implicated the NAcc in multiple aspects of drug processing including the approach to the operant (Chang et al, 1994; Nicola, 2010), operant

responding (Chang et al., 1994; Peoples et al., 1997; Sokolowski et al., 1998; Fabbricatore et al., 2010; Carelli and Deadwyler, 1993, 1994; Ghitza et al, 2004; 2006), and fluctuations in bodily concentrations of drug (Peoples et al., 1998; Peoples & West, 1996; Nicola & Deadwyler, 2000). Moreover, studies have suggested that changes in the tonic activity of accumbens neurons may change across protracted drug use (Peoples et al., 1999). Given that the NAcc has been implicated in a wide variety of behaviors, data were collected for 1) phasic firing during general locomotion 2) phasic firing during approach to the operant 3) phasic firing during the operant head movement 4) the rank-order correlation between firing rate and calculated drug level and 5) tonic firing rate. Each of these events was monitored during the transition from acute to chronic drug exposure.

Unless otherwise noted, event-related firing patterns were analyzed using the change ratio ΔFR_{event}=(Event - Baseline)/(Event + Baseline). Values for this ratio range between -1 and +1 with negative and positive values representing decreases or increases from baseline firing rate, respectively. Change scores served to normalize event related firing to baseline firing within-session, thereby allowing for congruent comparisons across sessions.

2.7.1- Baseline firing rate.

The NAcc has primarily been implicated in behaviors that involve some form of motor response (e.g., approach or operant responding). Therefore, movement-free epochs were used to calculate baseline firing rates as a control for possible influences of motor behaviors on NAcc firing. To determine movement-free epochs, a custom MATLAB

script was used to examine subjects' tracked coordinates across the entire session and extract moments when rats were still for a period of at least one second (pink tape moved less than 5 pixels, equivalent to 5.03 mm; Figure 4A). Timestamps for these periods were extracted and the number of discharges per sec during each period was used to calculate average firing rate during all movement-free intervals. Firing during movement-free periods will henceforth be referred to as 'baseline firing rate.' Comparisons between baseline firing rate and other methods for calculating a firing rate baseline (i.e. utilizing a pre-node period or a scrambled/randomly sampled baseline) revealed that all measures of baseline firing were highly correlated. Thus, while the movement-free baseline was specifically chosen as a control for the behaviors of interest, as a measure of baseline firing rate it was similar to any of the aforementioned canonical baseline measures.

Baseline firing rates also served as a matched set for the analysis of changes in NAcc firing over sessions. Pilot analyses demonstrated that firing rates obtained using this method correspond highly with measures of on-drug firing that our laboratory has obtained previously, e.g., "tonic firing rate", by sampling larger blocks of time during the maintenance phase of self-administration (data not shown; Peoples et al., 1999). In order to account for changes in behavior that might occur longitudinally, non-movement baseline firing rates were used in order to maintain behavioral equivalence when comparing across sessions. For analysis, baseline firing rates were included in their raw form (spikes/second).

2.7.2- Approach Tracking.

An approach was operationally defined as an overt, single-directional movement towards the response apparatus which resulted in the animal engaging the photo-beam device (Figure 2; Figure 4C). Animals' approaches to the photocell-corner (i.e. response apparatus) were determined using a custom MATLAB script. The algorithm was designed to find the timestamp corresponding to the initial photocell break for each operant response and then track backwards in time to the point at which the animal's distance from the photocell stopped increasing using the formula:

$$d_t = \sqrt{(x_{t2} - x_1)^2 + (y_{t2} - y_1)^2}$$

Where:

d = distance (pixels)

X_1 and Y_1= the animal's coordinates at the time of a photo-beam break (i.e. operant response).

Xt_2 and Yt_2 = the coordinates for the animal at each point prior to the operant response.

d_t was calculated for every t (time point) prior to the start of an head movement until $d_t \geq d_{t+1}$. t is then taken as the time point where the approach starts. A pixel corresponded to an approximate distance ranging from 0.69 mm to 1.06 mm, with the uncertainty owing to the inability to accurately measure distance in all three dimensions. Nonetheless, by tracking continuous motion backwards from the response, ignoring

distance per se, this procedure unambiguously determined the time point at which animals began their approach (Fig. 4).

In order to create matched sets of approaches across sessions, approaches were broken into categories based on 1) the distance of the approach and 2) the duration of the approach. Approach distances were determined by separating the chamber into four quadrants. Approaches were broken into long- and short-duration movements using a median split. Pilot analyses of the population of approaches showed that the median approach lasted 800 milliseconds. Therefore, values below 800ms were designated as short-duration approaches, while those above 800ms were designated as long-duration approaches. Overall, the combination of 4 levels of distance and 2 levels of duration produced 8 approach categories.

For all approaches, firing rate during the movement was compared to baseline firing rates within each session using the ratio '$\Delta FR_{Approach}$=(Approach - Baseline)/(Approach + Baseline)', where 'Approach' represents firing rate during a specific approach category and 'Baseline' represents the baseline firing rate (calculated as described in section 2.6.1). Change ratios were then analyzed across sessions.

2.7.2- General Locomotion-

While multiple studies have implicated the NAcc in goal-directed locomotion (i.e., approach to the operant), few studies have attempted to examine or rule out the relationship between NAcc firing and non goal-directed locomotor behaviors. Furthermore, comparisons between goal-directed locomotion and non-goal directed locomotion are also lacking.

In order to address these issues, nonspecific locomotor behaviors were extracted using a custom MATLAB script (Figure 4B). Instances of general locomotion were extracted by finding moments when subjects locomoted to the quadrant diagonally opposite to the photocell quadrant (henceforth termed the 'control quadrant'). Similar to approach tracking, the distance traveled was quantified from the beginning of each single-directional movement until the movement ended in the control quadrant. For general locomotor responses, it was required that the start of the movement was not preceded by a photocell break (i.e., was not a retreat from the operant). Locomotor behaviors were then matched to Approach behaviors by comparing mirrored movements into the photocell and control quadrant that were matched for movement distance and duration.

The firing patterns of NAcc neurons during general locomotion were examined by comparing firing during general locomotion to non-movement, baseline firing rates within each session using the ratio '$\Delta FR_{\text{General Locomotion}}$=(Locomotion - Baseline)/(Locomotion + Baseline)'. Similarly, goal-directed and non goal-directed locomotor movements were compared using the ratio '$\Delta FR_{\text{Goal Directed Locomotion}}$=/(Approach - Locomotion)/(Approach + Locomotion)'. Change ratios were then examined across sessions.

2.7.3- Operant Head Movements.

Phasic increases or decreases in NAcc firing during operant responding has long been reported (Chang et al., 1994; Peoples et al., 1997; Sokolowski et al., 1998; Fabbricatore et al., 2010; Carelli and Deadwyler, 1993, 1994; Ghitza et al, 2004; 2006).

To examine firing patterns during the operant response, head-movements (Figure 4D) were broken into 50 unique movement categories. These fifty categories correspond to a combination of five different categories of movement duration, at fourteen unique categories of movement start and end position (movement distance). Most importantly, categories represent similar movements with equivalent sensorimotor properties such that they can be 'matched' across sessions. Notably, categories cover a multitude of movements and not just the 'criterion response' for self-administration behavior.

For some animals, movements extended beyond the top-most photocell of the response apparatus. For these movements, a custom MATLAB script was used to detect the final ending coordinate (pixels) and final end time of the movement. The script utilized Y-coordinates from the video tracking to find the inflection point at which the upward response ended and shifted to a subsequent downward motion. Movements that extended beyond the photocell device were filtered out of the movement database in order to retain the photocell device's accuracy in measuring movement distance, duration, and starting and ending times.

Movements that were maintained across sessions (i.e. matched sets) were compared to baseline firing rates within each session using the ratio '$\Delta FR_{response}$=/(Response - Baseline)/(Response + Baseline)'. Change ratios were then examined across sessions.

2.7.4 Correlation of NAcc firing and Drug Level.

Previous work from our laboratory (Peoples et al., 1998; Peoples & West, 1996) and others (Nicola & Deadwyler, 2000) has demonstrated that a relationship exists

between NAcc firing rate and calculated drug level. Still, to the best of our knowledge, it has yet to be determined whether or not this relationship is inherent (i.e., strictly pharmacological), or conditioned and thus develops over sessions. To address this, firing rate and calculated drug level were correlated within each session by creating bins of calculated drug level representing each 0.5 mg/kg increment from 0 to 5 mg/kg. Based on pilot analyses of the data, a Spearman's rank order correlation was chosen in order to handle deviations from normality. The rank order correlation sufficiently handles linear and non-linear relationships between drug-level and firing rate and is highly correlated with the Pearson's correlation coefficient when considering linear data. Correlations of firing rate and calculated drug level were determined for each session, and then the strength of this relationship was examined across sessions by analyzing the Spearman's rho coefficient longitudinally.

2.7- Statistical Analyses of Neural Data:

Analyses of the neural firing patterns for the above mentioned behavioral events were conducted using a linear mixed model in SAS PROC GLIMMIX (SAS Institute Inc., 2005, Cary, NC). All models included 12 levels of recording (blocks of every 2 training sessions for 24 sessions) and 3 levels of subregion (i.e., Core, Shell, and Core/Shell border). For all models, recording session was designated as a continuous variable, while subregion was incorporated as a categorical variable. Behaviors for which no effect of subregion *and* no subregion x recording interaction was observed were subsequently run using a simplified, one-level model (i.e., the main effect of recording session) in order to retain power and balance Type I and Type II errors. Post-hoc

comparisons for each model were Sidak-Holm adjusted to control for inflated Type I error via an inflated familywise error rate. For all tests alpha was set to 0.05.

Models were run for a total of 6 dependent measures. First, baseline firing rate was examined across sessions and between subregions. Second, firing patterns for behavioral events were examined by modeling change scores for general locomotion, approach to the operant, and operant head movements as compared to baseline firing rate. Third, differences between goal-directed approach and general locomotion were examined by modeling the change in firing for approach behaviors relative to matched sets of general locomotion. Finally, the correlation of drug level and firing rate was modeled across sessions and subregions to investigate whether or not accumbens neurons exhibited changes in drug-related firing during the transition from acute to chronic drug use. Post-hoc comparisons for change-scores and the rank order correlation were made at the level of each recording session and designed to examine whether each value differed from a value or zero, representing no change.

3—Results

3.1—Self-Administration Behavior

Self-administration behaviors are shown in Figure 5. All subjects demonstrated evidence of learning across self-administration training. Animals significantly increased the number of emitted criterion head movements—operationally defined as sequentially breaking photocells 2 through 5 within one second—from 37.8± 5.6 movements in session 1, to 168.9 ± 17.1 movements in session 24 [$F(1,20) = 37.71$, $p < 0.001$; $\beta = 5.17$]. Accordingly, this corresponded to a significant increase in the number of earned rewards

from 21.7 ± 3.7 in session 1 to 89.6 ± 9.2 earned rewards in session 24 [F (1, 20) = 50.24, $p<0.001$; $\beta= 2.62$].

Animals also demonstrated evidence of skill learning across sessions. Although no changes were observed in movement distance across sessions (Session 1: 52.6 ±3.9 mm; Session24: 53.2 ± 1.4 mm; $\beta= 0.02$), criterion movements became faster across sessions (Session 1: 0.38 ± 0.06 s; Session 24: 0.17 ± 0.03s; [F (1,20)=15.93, $p < 0.001$; $\beta= -0.007$]) resulting in a significant increase in movement velocity from 235.8 ± 41.5 mm/sec in Session 1 to 432.9± 50.3 mm/sec in Session 24 [F (1,20) = 14.23, $p<0.01$; $\beta= 6.93$].

Finally, all animals demonstrated an escalation of drug intake across sessions. Drug consumption significantly increased from 10.5 ± 0.6 mg/kg in Session 1 to 23.5 ± 1.7 mg/kg in Session 24 [F (1,20) = 51.75, $p<0.001$; $\beta= 0.48$]. This escalation may be partially explained by a significant decrease in bodyweight over sessions (Session 1: 336.2 ± 2.3 grams; Session 24: 319.3 ± 2.9 grams; F[(1,20)=41.23, $p< 0.001$; $\beta= -0.84$]).

3.2—Histological Results and Waveform Properties

To be included for longitudinal analysis, neurons were required to be present for a minimum of 8 out of 24 intermittent recording sessions (i.e., 4 recordings). Moreover, it was required that neurons maintained at least a 2:1 signal: noise ratio and showed no discharges during the neuron's natural refractory period (~1.4 - 2.0 ms). Finally, it was necessary that waveform parameters including the spike height, and the time of valley and peak voltage remained stable across all sessions (i.e. showed no greater than a 20% change).

Of 336 wires implanted into the basal forebrain, 147 wires from 14 animals were localized within the nucleus accumbens. Sixty-eight of these wires exhibited a single-unit that was maintained for longitudinal analysis (Figure 6; Core=30; Shell=29; Core/Shell border=9). Recordings from these neurons yielded a total of 625 'neuron sessions'. Animals were included for neural analysis only if 1) The anatomical placement of each recording probe could be definitively identified 2) recording probes were localized within the NAcc and 3) A NAcc probe held a neuron for a minimum of four recording sessions (~8 days). Animals whose data did not meet the criteria to be included in neural analyses were still included in behavioral analyses.

For neurons held longitudinally, the average neuron was maintained for 8.86 ± 0.29 recordings, which roughly corresponds to 17.36 ± 0.58 Sessions. Notably, recordings span the entire range of sessions with neurons that were present in late as well as early sessions, neurons that were present in late but not early sessions, neurons that were present early but not late sessions, or neurons for whom intermittent recordings might have been missed to due temporary hardware failures etc. Also, independent samples t-tests revealed that there was no difference in the number of recordings between NAcc subregions (core, shell, and dorsal border; all $p > 0.14$, NS).

Nearly all waveforms exhibited an initial negativity in the waveform. This is consistent with previous extracellular recordings of striatal medium spiny neurons from our laboratory (Tang et al., 2007). The average signal: noise ratio for neurons recorded from the accumbens was 4.23 ± 0.12 : 1. An independent samples t-test revealed no differences in the signal: noise ratio between the core, shell, and dorsal border (all, $p > 0.11$, NS).

The average waveform amplitude for core neurons was 109.651 ± 4.48μV. The amplitude of Shell neurons was 112.65 ± 6.97 μV. Finally, the amplitude of neurons localized to the dorsal border was 183.88± 44.33 μV. These values are consistent with the range of amplitudes for NAcc medium spiny neurons that we have reported previously (Peoples & West, 1996). An independent samples t-test revealed no differences between the amplitude of core and shell neurons (all $p > 0.13$, NS).

For extracellular recordings, the time from waveform valley to peak was 105.41 ± 5.51 μS in the core, 106.13 ± 5.07 μS in the Shell, and 82.63 ± 21.97 μS for neurons on the dorsal border. No differences were observed in the latency from valley to peak between subregions (all $p > 0.15$, NS).. All waveform characteristics are summarized in Table 1.

3.3—Neural Results

Average change scores for all modeled events are summarized in Table 2. The data are also represented graphically in Figure 7 for the core, shell, and core/shell border (also see Appendix A).

3.3.1- Baseline Firing rate.

Initial analyses of baseline firing rates revealed two neurons which were flagged as outliers. Both neurons were greater than 4.6 standard deviations from the population mean. The average firing rate for these neurons was 19.66 ± 3.4 spikes/second, which is well above the average firing rates we have reported previously for accumbens neurons (0.53 ± 0.12 spikes/sec; Fabbricatore et al., 2010) and well above the population average in the present study (0.49 ± 0.65 spikes/sec; outliers included). Thus, both outliers were

removed from analyses of baseline firing rate and all subsequent analyses. Indeed, while medium spiny projection neurons are known to constitute >95% of all striatal neurons, physiological evidence has also identified multiple types of faster firing interneurons which are outside the scope of the present study (Kreitzer, 2009) and of which these outliers are likely representative (~3%; 2 of 70 neurons).

Baseline firing rates averaged 0.32 ± 0.06 spikes/second during the first recording session and slowly increased to 0.74 ± 0.26 spikes per second by the final recording session. Analyses of baseline firing rates showed no evidence for change across sessions (main effect of session: $p=0.14$), nor were any differences in baseline firing rate observed between subregions (main effect of subregion: $p=0.36$; recording session x subregion interaction: $p=0.69$). The average baseline firing rate over all sessions for neurons in the Nucleus Accumbens shell was 0.43 ± 0.03 spikes/second. Core neurons baseline firing patterns averaged 0.46 ± 0.04 spikes/second. Finally, neurons localized on the core/shell border exhibited an average firing rate of 0.43 ± 0.04.

3.3.2- General Locomotion & Approach Behaviors.

The average duration for matched general locomotor behaviors and approaches was 1.75 ± 0.16 s and 1.44 ± 0.22 s, respectively. Locomotor and approach behaviors ranged between 266 and 2469 ms in duration. No evidence was observed for differences between subregions nor any interaction of subregion and recording session for either general locomotion and approach behaviors (all $p > 0.20$). Thus, the statistical model was simplified to a one level model with subregions collapsed.

Single-level models for approach and general locomotion showed that firing

during approach and general locomotion was not different from baseline firing rates (figure 7; Appendix A) and did not change across recording sessions (Intercept: General Locomotion- $p = 0.42$; Approach-$p = 0.22$; Main effect of recording session: General Locomotion- $p=0.41$; Approach- $p =0.68$).

When approach-related firing was examined relative to general locomotor behaviors, a significant effect of recording session was observed [$F(1,60) = 4.44$, $p =0.04$] along with a significant intercept for the linear model [$t (60) = -2.87$, $p < 0.01$]. Sidak-holm corrected post-hoc comparisons revealed only a marginal decrease in firing during approach behaviors when compared to general locomotion during the first three sessions [Sessions1-3: all $t(60) > -2.70$, $0.09> p >0.05$, N.S.]. Thus, a subtle decrease in firing was observed during the approach when compared to general locomotion during early sessions.

3.3.3- Operant Responding.

Matched operant responses averaged 274.6 ± 0.45 ms in duration and exhibited an average distance of 32.25 ± 0.03 mm. On the average, matched movements were significantly shorter than the 'criterion response' ($p<0.05$) . Included matched movements did, however, cover the entire range possible starting and ending positions for movements emitted in the photocell apparatus, which are all interpreted as drug-seeking responses.

Neurons typically exhibited decreases in firing during the operant response relative to baseline (Table 2; Figures 7,8). However, the magnitude of response-related decreases became less pronounced over recording sessions. Similar to models of approach and general locomotion, the two level model for operant head movements and

firing rate revealed only a marginal effect of subregion [$F(2,305) = 2.82$, $p = 0.07$ N.S.] and a non-significant region by recording interaction ($p = 0.10$). Marginal differences between regions originated from differences between the average change score for core neurons (-0.35 ± 0.06) when compared to the average change score for neurons localized to the core/shell border (-0.21 ± 0.14).

Subsequent analyses on a reduced, one-level model (subregion collapsed) revealed a significant increase in response related change scores across recording sessions [$F(1,62) = 6.83$, $p = 0.01$] and a significant negative intercept [$t(62) = -6.10$, $p < 0.001$]. Post-hoc comparisons revealed that firing during the operant response was significantly decreased relative to baseline firing rate during all twelve recording sessions [Recording sessions 1-24: all $t(62) < -2.65$, $p < 0.01$].

3.3.4-Drug level and Firing Rate.

Neurons typically exhibited a negative correlation of firing rate and calculated drug level. Correlation coefficients averaged -0.23 ± 0.06 during the first session and became slightly more negative over training, averaging -0.37 ± 0.07 on session 24. The two-level model of drug level revealed no main effect of region ($p = 0.54$) nor a significant interaction of recording session x region ($p = 0.17$).

The simplified one level model suggested that the relationship between firing rate and drug level did not change across recording sessions (main effect of recording sessions: $p = 0.21$), but did reveal a significant intercept [$t(65) = -3.65$, $p < 0.0001$]. In combination, these results suggested that Spearman's rho values were stable across sessions, although significantly different from zero (no correlation). This was confirmed

by post-hoc comparisons, which revealed that Spearman's rho values were significantly below zero for all recording sessions[recording sessions 1-24: all $t(65) \leq -3.65$, $p < 0.0001$]. These data suggest an unconditioned negative correlation between drug level and firing rate. Thus, throughout self-administration training, increases in drug level typically resulted in decreases in the firing rate of accumbens neurons (figure 9).

4—Discussion

It has long been shown that the effects of psychomotor stimulants are dependent on the nucleus accumbens (NAcc) and its dopaminergic and glutamatergic afferents (Pettit et al., 1984, Roberts et al., 1977). Moreover, studies have shown that changes occur in the ventral striatum during the transition from acute drug exposure to chronic drug use (Letchworth et al, 2001; Macey et al, 2004; Porrino et al, 2004; Nader et al., 2002). Nevertheless, previous studies have yet to address how this transition affects functional processing in the Nucleus Accumbens. Results from the present study establish that continued cocaine use corresponds to both stable pharmacological effects on the firing patterns of nucleus accumbens neurons and dynamic changes in the firing patterns of accumbens neurons during operant responding. Firing during locomotor behaviors and goal-directed approaches, however, was not different from baseline firing rates.

4.1- Acquisition of skilled cocaine self-administration.

Animals in the current experiment escalated their intake of cocaine over self-administration sessions and learned the complex head-movement operant. The escalation of drug intake over long-access sessions is critically important for modeling neural

changes across protracted drug use, as escalated drug intake has been touted as the preclinical analog to human cocaine dependence (Ahmed and Koob, 1998).

Similar to previous reports from our laboratory (Root et al., 2011; Root et al., 2013), it was also shown that animals exhibited skill learning for the complex operant head movement. Beyond increases in the number of daily criterion responses, animals demonstrated increases in movement velocity and a concomitant decrease in movement duration. While not required, this suggests that animals became more efficient at operant head movements over sessions.

4.2-Unconditioned effects of cocaine concentration on the firing patterns of Accumbens neurons.

Based on evidence that morphological changes occur in accumbens neurons following protracted drug use (e.g., Waselus et al., 2013), it was predicted that cocaine's effect on the firing patterns of accumbens neurons would change over time. Instead, the firing of accumbens neurons was consistently inhibited by cocaine across sessions. This inhibition became more pronounced as calculated levels of cocaine increased *within* each session, although the observed relationship was not always linear. Negative correlations *across* recording sessions, however, were stable for the sample of longitudinally recorded cells. Individual neurons did show both increases and decreases in correlative strength. Nevertheless, the majority of observed correlations remained negative across all recording sessions.

Previous work has demonstrated that NAcc firing patterns are correlated with changes in calculated drug level (Peoples et al., 1998; Peoples & West, 1996; Nicola &

Deadwyler, 2000). Still, it remained unclear whether these relationships developed over sessions or if they represented an inherent effect of cocaine on neural firing patterns. Based on the present results, it might be suggested that the relationship between calculated drug levels and cocaine does not develop over sessions, but is instead an inherent pharmacological effect of the drug on the firing of accumbens neurons.

Despite the fact that the average across the sample of recorded neurons did not change across recording sessions, changes that did occur in individual neurons should not be discounted. Indeed, changes in dendritic spine density have been shown to occur following repeated cocaine experience (Waselus et al., 2013) and increases in AMPA receptor expression have been observed following sensitization to cocaine (Bordreau & Wolf, 2005). Thus, changes in individual neurons' response to cocaine over sessions may reflect some form of underlying neuroplasticity.

The heterogeneity of changes observed in the relationship between firing rate and drug level among individual neurons (i.e., increases and decreases over sessions in the same neuron's firing rate) may also reflect differences among subtypes of accumbens medium spiny neurons. For example, accumbens neurons are known to express either the neuropeptides substance-p and dynorphin or the neuropeptide enkephalin (Kelley et al., 2005), and these neuropeptides are known to be affected differentially following cocaine self-administration (Hurd et al 1992). Alternatively, these differences may correspond to D1- and D2-receptor expressing striatal neurons, which have also been shown to respond differentially to cocaine administration (Bertran-Gonzales et al., 2008).

4.3- Firing patterns of Accumbens neurons during operant head movement shift dynamically over time.

Nearly all accumbens neurons exhibited decreases in firing rate during the operant response. Moreover, the magnitude of the observed decreases became attenuated over recording sessions. This result is consistent with previous work showing that the firing of accumbens neurons is modulated around the operant response. Chang et al., 1994; Peoples et al., 1997; Sokolowski et al., 1998; Fabbricatore et al., 2010; Carelli and Deadwyler, 1993, 1994; Ghitza et al, 2004; 2006. The present study extends these results, however, by demonstrating that response related firing patterns change across protracted drug administration and across task learning. Moreover, while previous studies have demonstrated that response sensitive neurons are present during single-session recordings, the present data demonstrated that the operant response is the only behavioral component of the present task for which neurons consistently exhibit a response over sessions.

It was predicted based on theories of habitual drug-seeking behavior that the accumbens would no longer be involved in drug-seeking behaviors late in training. While it did appear that response-related firing patterns were attenuated over time, results suggested that the accumbens continued to play a role in drug seeking. Indeed, response-related firing patterns persisted in spite of twenty-four sessions of long-access drug administration. Thus, the present data do not support the prediction that the accumbens is no longer involved in processing drug-seeking behaviors following protracted drug use.

The change in the magnitude of response related firing over sessions may be accounted for by a number of theories of accumbens function. First, the decreased magnitude of response related firing may represent changes in synaptic efficiency. Accumbens projection neurons are typically GABAergic and therefore affect downstream

targets via inhibition or the release thereof (Kelley et al., 2005). Given that response related firing decreased in magnitude, but remained different from baseline across twenty-five sessions of self-administration training, it might be suggested that accumbens neurons were able to enact movement related changes via smaller and smaller amounts of downstream disinhibition as training progressed.

The change in response related firing over sessions might also suggest that the accumbens is most involved during learning and becomes less involved as task performance stabilizes. Studies have shown that the accumbens is necessary for response-reinforcement learning (Chang et al., 1994; Peoples et al., 1997; Sokolowski et al., 1998; Fabbricatore et al., 2010; Carelli and Deadwyler, 1993, 1994; Ghitza et al, 2004; 2006). In accordance with these results, animals in the present study also exhibited response-specific decreases in firing and showed no relationship between the accumbens firing and locomotion. Studies have also shown that dopamine release in the accumbens relates to the number of emitted lever presses and *not* the number of pellets delivered or the consumption of food rewards (Sokolowski et al., 1998). Overall, these results suggest a somewhat specific role for the accumbens in the functional processing involved in response-reinforcement learning in lieu of a more general function in reward processing, motivation, or arousal.

4.4-Accumbens firing patterns during general locomotion and approach.

Accumbens neurons did not exhibit significant increases or decreases in firing rates during locomotor behaviors or goal-directed approaches to the operant. Given that multiple studies have reported firing during goal-directed approach (Chang et al, 1994;

Nicola, 2010), the absence of approach related firing was perhaps the most surprising result of the present study. Nevertheless, the absence of firing patterns during approach, together with the concomitant presence of altered firing during the operant response itself suggests that the response of accumbens neurons in the present task is specific in nature.

A number of factors may account for differences between previous studies and the present data. First, approach related firing may change depending on the behavioral task. Given the presence of firing during the operant response in the present study, one might speculate that the same effect might be observed during the approach for tasks where the approach represents a greater portion of the reward-seeking response (e.g., approach to the reward in a pavlovian task).

Alternatively, approach related firing is sometimes defined as an arbitrary window prior to the response (e.g. 1 second) rather than by extracting the starting and ending times of individual locomotor responses. Notably, previous work in our laboratory had reported that phasic increases or decreases in accumbens firing around the operant response typically occur within -1.5 and +1.5 seconds around the operant response (Ghitza et al., 2004). An analysis of the present data which applied this same window to all neurons (results not shown) suggested that the observed changes in firing rate reflect a hybrid of approach and operant movement related firing. Thus, observations of approach-related firing in other studies may actually reflect a blend of approach and response-related firing.

4.5- Concluding Remarks: Integrating the present data into theories of striatal function

Theories of addiction have suggested that long-term changes in striatal activity may be responsible for the transition from goal-directed drug seeking behaviors to automatic or habitual responding (Everitt and Robbins, 2005). Specifically, these theories suggest that action-outcome learning early in training relies on the nucleus accumbens while habitual (i.e., stimulus-response) learning relies on more dorsal portions of the striatum. The central tenet of this model is that processing in the accumbens is no longer necessary once responding becomes reliant on stimulus-response relationships, or that stimulus-response processing in the dorsal striatum increases and dominates behavioral output (Everitt and Robbins, 2005).

The present data provide little support for the theory that habitual drug seeking develops following chronic drug use. Indeed, while the magnitude of decreases for response-related firing was attenuated late in training, the population of accumbens neurons exhibited task related processing across all 24 long-access training sessions (12 recordings). Therefore, we would posit that accumbens neurons retain a role in processing behavior following extended training. Moreover, data collected in parallel with the present experiment for response-sensitive dorsolateral striatum neurons in this same task suggested that those neurons also show a decrease in the magnitude of response-related increases as training progresses (unpublished observations). Thus, the prevailing evidence does not fully support predictions made by theories of habitual processing in addiction.

The above mentioned similarities in ventral and dorsal striatal processing during the current task are perhaps important, however, when considering the role of the accumbens in striatal functioning as a whole and its role in behavioral output. Given the

dense anatomical projection from the accumbens to the ventral pallidum (Zahm & Heimer, 1990) studies often consider the ventral pallidum to be the accumbens' primary output nucleus. This notion is in line with recent data from our laboratory demonstrating response-related firing in the ventral pallidum (Root et al., 2013). Thus, it is perfectly plausible that the accumbens affects behavioral output via striatopallidothalamocorical connections. Observed trends in the ventral pallidum did, however, contrast with the present results in that pallidal neurons exhibited modulations of firing during both responses and goal-directed approaches to the operant.

Anatomical evidence has also suggested a second route by which the accumbens might eventually affect behavior which involves multiple sequential connections from the ventromedial striatum to the dorsolateral striatum (Haber et al., 2000). These ascending connections originate in the accumbens shell, and project to medial portions of the ventral tegmental area (VTA). Medial VTA neurons then project to the more lateral accumbens core, which then engages more lateral portions of the VTA, as well as portions of the substantia nigra. In continuing fashion, reciprocal nigrostriatal connections engage the medial portions of the dorsal striatum and then progressively more lateral portions of the substantia nigra and dorsal striatum (Haber et al., 2000).

Given the response-related firing of accumbens neurons, and the concomitant response-related firing we have observed in the dorsolateral striatum during this same task (unpublished observations), the present results provide putative support for such an ascending anatomical spiral, with signaling occurring over a rapid time scale (i.e., on the order of milliseconds). Thus, the observed similarity between core and shell neurons in the present task may represent the first stages of an ascending anatomical circuit in which

inhibited GABAergic neurons in the accumbens shell eventually come to inhibit neurons in the accumbens core via an intermediate projection to GABAergic neurons in the VTA (as predicted by Haber & colleagues, 2000). And, as described above, the response-related increases in dorsolateral striatum firing during the present task could involve a disinhibition via the laterally spiraling mesencephalic-striatal connections.

Overall, this theory would suggest a unified role for the striatum in response-reinforcement learning and perhaps provides a framework through which a number of disparate theories of striatal involvement in 'reward', 'effort', 'motivation' etc. might be unified. Furthermore, this theory of ascending striatal connections need not be mutually exclusive from theories of striatopallidal signaling. Instead, the two may work synergistically in order to carry out specific motivated behaviors.

When combining the current evidence with studies showing morphological changes in accumbens' neurons (Waselus et al., 2013) and changes in accumbens' excitability (Nader et al., 2002) following extended drug administration, one might suggest that dynamic changes in the accumbens' response-related firing may represent changes in synaptic efficiency. That is, the magnitude of the neuronal response from accumbens neurons to produce a behavioral output may decrease over extended training. This type of increase in the excitability of accumbens' neurons could contribute to response perseveration by facilitating behavioral output and/or by producing deficits in response inhibition. Moreover, these types of changes may also contribute to the likelihood of relapse should they persist during periods of abstinence.

4.6 Suggested Future Directions

The current study provides a framework for the type of longitudinal work that is necessary to understand changes in neural activity which correspond to the development of substance dependence. These data focus on population trends observed in the firing of accumbens neurons over time. Indeed, the morphology of the striatum with homogeneously spaced neurons with small amplitude signals is conducive to this type of study, as it allows for individual neurons to be effectively tracked over time. Moreover, the growth-curve style modeling used in the present study is a powerful tool for examining population trends while retaining information about individual neurons.

While the present data effectively capture population trends, future work might build from these data by expanding upon the present analyses. First and foremost, there are notable differences in animals' behavior early in training as compared to late in training. These differences may be of specific importance in the development of substance dependence and could be examined by focusing on the dynamic changes that occur early in training or focusing on changes that occur just prior to or after the escalation in drug consumption. In other words, focus should be given to specific epochs or blocks of time across the training period. Indeed, these more acute changes are not specifically captured by the current population level analyses. Further analyses might also focus on variability amongst neurons. Certainly, there is some variability in the magnitude or direction of change observed amongst neurons longitudinally. Data reduction techniques (e.g. cluster or factor analyses) might be used in order to classify these neurons and focus analyses on neurons that change similarly over time. Alternatively, it might be especially effective to implement cell-type specific targeting

and optogenetics in order to identify specific neural populations that could be tracked longitudinally in order to determine the respective contributions of specific populations.

Longitudinal analyses might also be extended to examine the firing patterns of accumbens neurons during operant responding during acute extinction/withdrawal or during tests of reinstatement. By examining response-related firing during probe sessions of acute extinction and withdrawal, future longitudinal studies might gain insight into the relative contributions of behavioral and pharmacological components of self-administration to the trends observed in the present study. Moreover, studies of relapse or reinstatement would provide important information about how the changes that occur during self-administration persist across abstinence in order to drive subsequent bouts of drug-seeking behavior.

Delving further into the source of the changes observed in the present data will help to answer a number of important questions raised by this first-of-its-kind longitudinal study. Perhaps most important amongst these is determining the mechanism by which response-related inhibition of accumbens neurons occurs. Given their proximity to a goal-directed behavior, it stands to reason that the firing patterns observed in a well-trained, skilled, and substance dependent subject would reflect underlying changes that could become the target of effective therapies.

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10. Tables and Figures

Table 1

Neuron Characteristics

	Core	Shell	Dorsal Border
N	30	29	9
Days Maintained	19.30 ± 0.68	20.33 ± 0.64	17.92 ± 1.41
No. of Recordings	8.39 ± 0.44	8.63 ± 0.43	9.58 ± 0.78
Signal: Noise	4.01 ± 0.14 : 1	4.42 ± 0.21 : 1	4.36 ± 0.38 : 1
Amplitude	767.57 ± 31.3 mV	788.56 ± 48.8 mV	1287.15 ± 310.3 mV
Pre-Amplification	109.65 ± 4.5 µV	112.65 ± 6.9 µV	183.88 ± 44.3 µV
Valley-to-Peak Latency	105.41 ± 5.51 µs	106.13 ± 5.07 µs	104.28 ± 4.06 µs
Firing Rate (Spikes/Second)	0.46 ± 0.04	0.43 ± 0.03	0.43 ± 0.04

Table 2

Recording Session	Event					
	Baseline Firing Rate	General Locomotion [1]	Approach: General Locomotion [2] α ǂ	Approach [1]	Operant Response [1] α ǂ	Spearman's Rho ǂ
1-2	0.32 ± 0.06	0.03 ± 0.034	-0.1 ± 0.03 §	-0.05 ± 0.04	-0.41 ± 0.07 *	-0.23 ± 0.06 *
3-4	0.36 ± 0.05	0.03 ± 0.04	-0.09 ± 0.03 §	-0.05 ± 0.04	-0.39 ± 0.06 *	-0.24 ± 0.06 *
5-6	0.40 ± 0.05	0.02 ± 0.03	-0.08 ± 0.03 §	-0.04 ± 0.03	-0.36 ± 0.05 *	-0.25 ± 0.05 *
7-8	0.43 ± 0.06	0.02 ± 0.03	-0.07 ± 0.03	-0.04 ± 0.03	-0.34 ± 0.05 *	-0.27 ± 0.04 *
9-10	0.47 ± 0.08	0.01 ± 0.03	-0.06 ± 0.03	-0.04 ± 0.03	-0.31 ± 0.04 *	-0.28 ± 0.04 *
11-12	0.51 ± 0.11	0.01 ± 0.03	-0.04 ± 0.03	-0.04 ± 0.03	-0.29 ± 0.04 *	-0.29 ± 0.04 *
13-14	0.55 ± 0.13	0.00 ± 0.04	-0.03 ± 0.03	-0.04 ± 0.03	-0.27 ± 0.04 *	-0.30 ± 0.04 *
15-16	0.59 ± 0.16	-0.00 ± 0.04	-0.02 ± 0.03	-0.03 ± 0.03	-0.25 ± 0.04 *	-0.32 ± 0.04 *
17-18	0.63 ± 0.18	-0.01 ± 0.04	-0.01 ± 0.03	-0.03 ± 0.03	-0.22 ± 0.04 *	-0.33 ± 0.05 *
19-20	0.66 ± 0.21	-0.01 ± 0.04	-0.00 ± 0.04	-0.02 ± 0.03	-0.20 ± 0.05 *	-0.34 ± 0.06 *
21-22	0.70 ± 0.24	-0.01 ± 0.05	0.01 ± 0.04	-0.03 ± 0.04	-0.18 ± 0.05 *	-0.36 ± 0.06 *
23-24	0.74 ± 0.26	-0.02 ± 0.05	0.02 ± 0.04	-0.03 ± 0.04	-0.16 ± 0.06 *	-0.37 ± 0.07*

[1]-Change score relative to baseline: (Event FR-Baseline FR) / (Event FR +Baseline FR)

[2]-Change score relative to general locomotion: (Approach FR - General Locomotion FR) / (Approach FR + General Locomotion FR)

α -Significant effect of recording session (significant model slope)

ǂ - Significant model intercept

* - Significant post-hoc for session when compared to expected value of 0 (no effect)

§-Marginally Significant post-hoc for session (0.10 > p > 0.5) when compared to expected value of 0 (no effect)

Figure 1

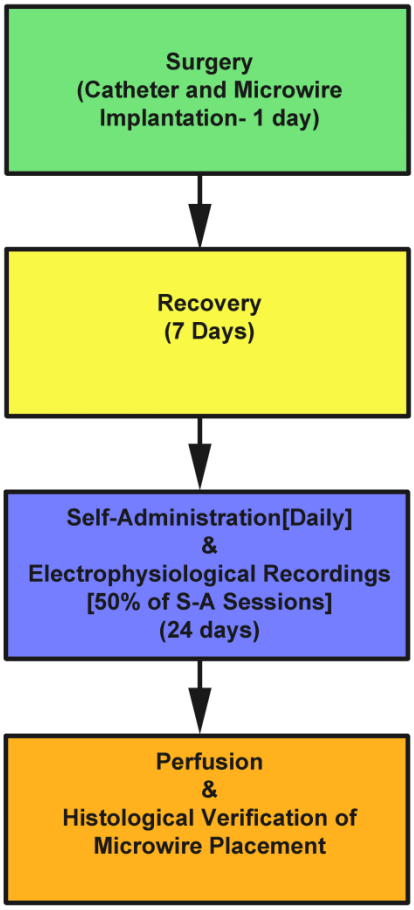


Figure 1. Experimental flow chart illustrating the order and timing of surgical procedures, recovery, self-administration training, and histological procedures.

Figure 2

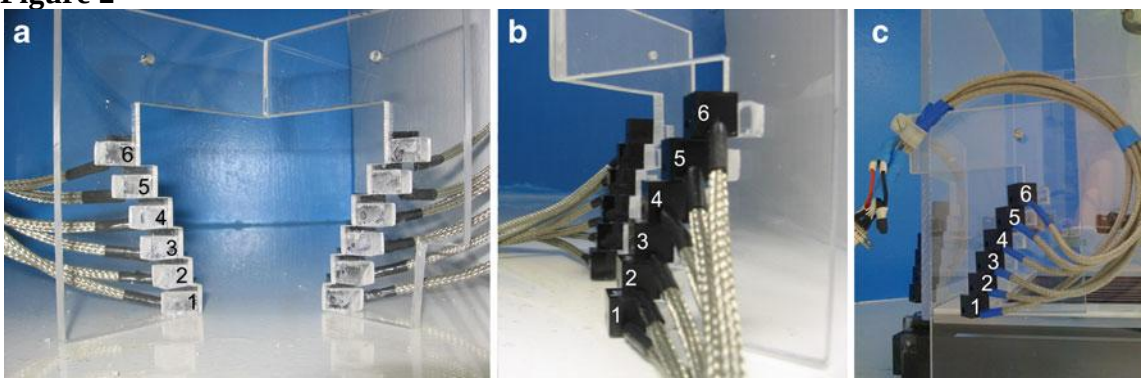


Figure 2. Images depicting the photocell apparatus used to detect operant head-movements. A) Front view of the device B) Side view showing photocells 1-6. Animals are required to consecutively break photocells 2-5 within 1 second in order to complete an operant response. C) Image of the photocell apparatus while attached to the back left corner of the conditioning chamber.

Figure 3

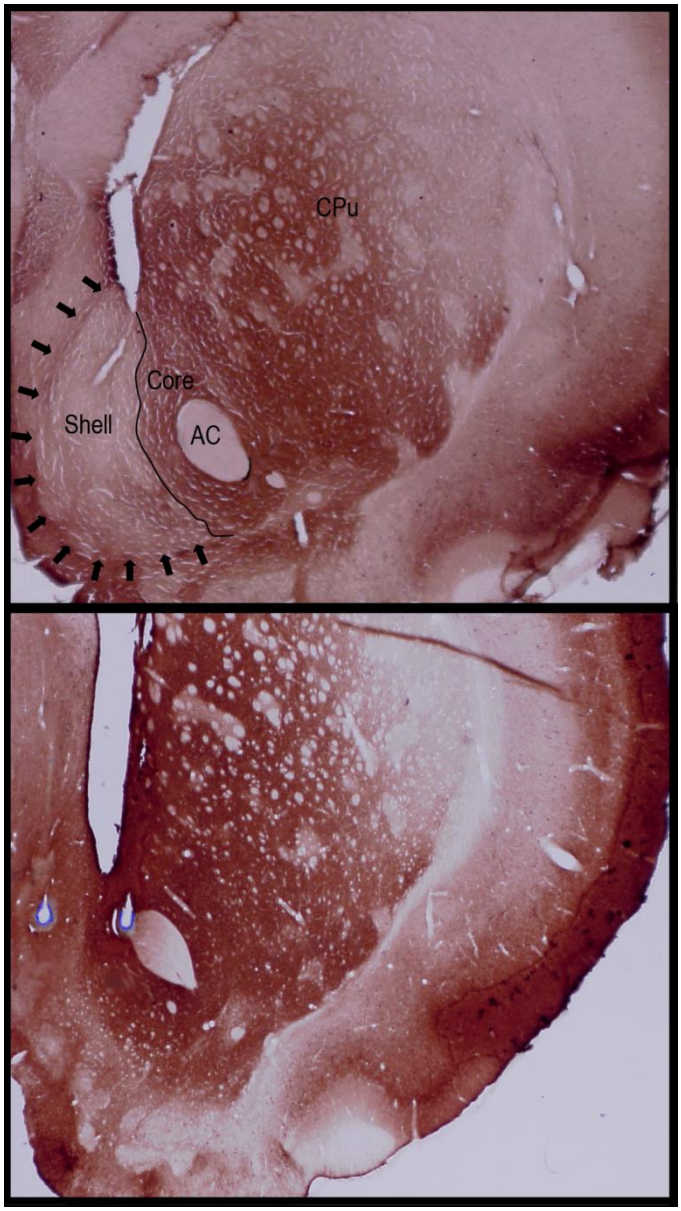


Figure 3. Histological delineation of the NAcc core and shell. *Top:* Calbindin D28-k reveals the Calbindin-poor NAcc shell as well as the Calbindin-rich NAcc core (AC-Anterior Commissure). *Bottom:* Lesions representing the location of microwires in the NAcc shell (left) and core (right) are stained blue using a potassium ferrocyanide stain.

Figure 4

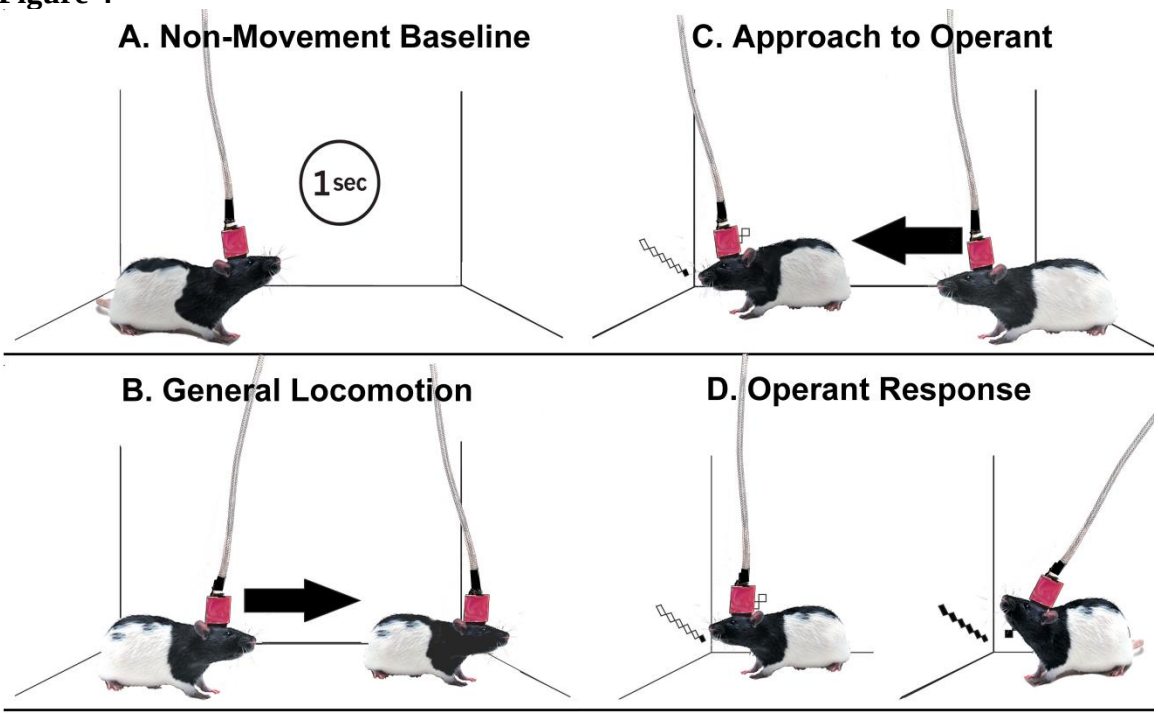


Figure 4. Illustration of behavioral events. Video tracking of behaviors was accomplished using the pink color tape on animals' recording harnesses which contrasted against a blue-background (not shown). A) Baseline firing rate was extracted during moments when animals were still for ≥ 1 second. B) General locomotion was defined as an epoch when the animal locomoted towards the front right corner of the chamber (away from the operant photocell-apparatus). General locomotor responses were selected to mirror animals approach to the operant and detected using video tracking C) Animals' approach behavior was defined as any single-directional movement which resulted in a photo-beam break at the operant. Approach behaviors were detected using video tracking D) Animals were required to perform a vertical, upward head movement through a series of photocells in the corner. To ensure the accuracy for measurements of movement distance and duration, video tracking was used to identify and eliminate movements which extended beyond the photocell device.

Figure 5

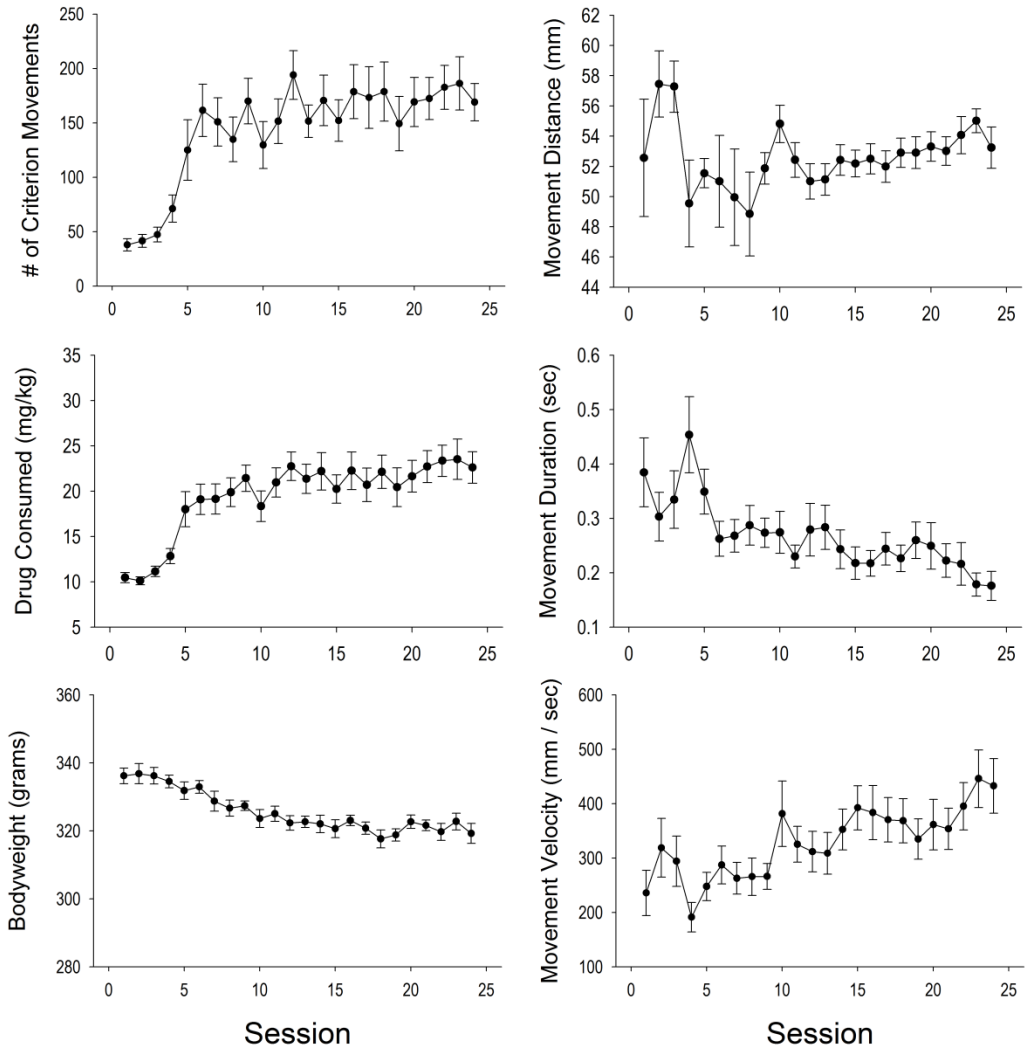


Figure 5. Relevant behaviors tracked across sessions. Session is shown on the X-Axis, while dependent measures are represented on the Y-axis.

Figure 6

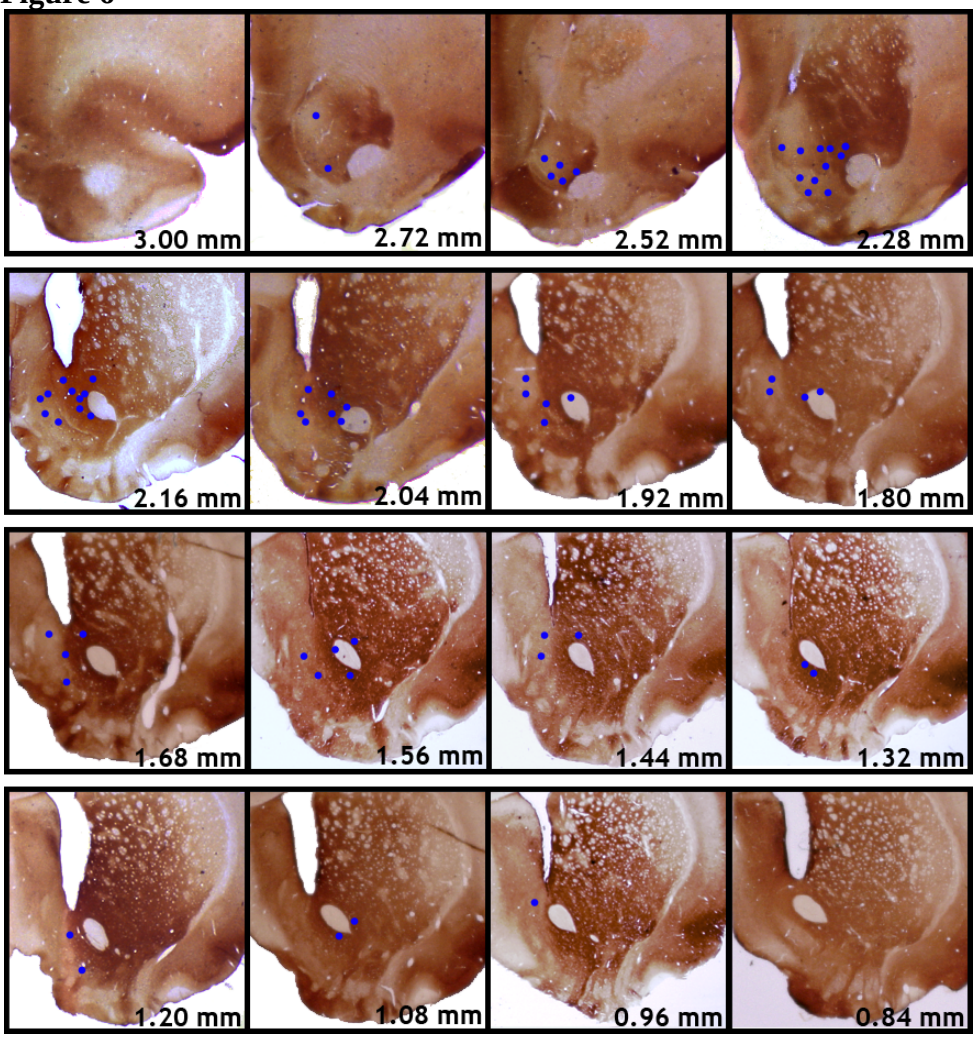


Figure 6. Representative histology for tissue stained for antibodies against Calbindin D28-K. Sections of tissue cover the entire anteroposterior extent of the nucleus accumbens and, for reference, align with plates from commonly used histological atlases for the rat (e.g. Paxinos & Watson, 1997). Relative locations are shown for microwires that 1) Were localized to the Nucleus Accumbens core, shell or core/shell border and 2) exhibited a single unit which was held longitudinally (minimum of 4/12 recording sessions).

Figure 7

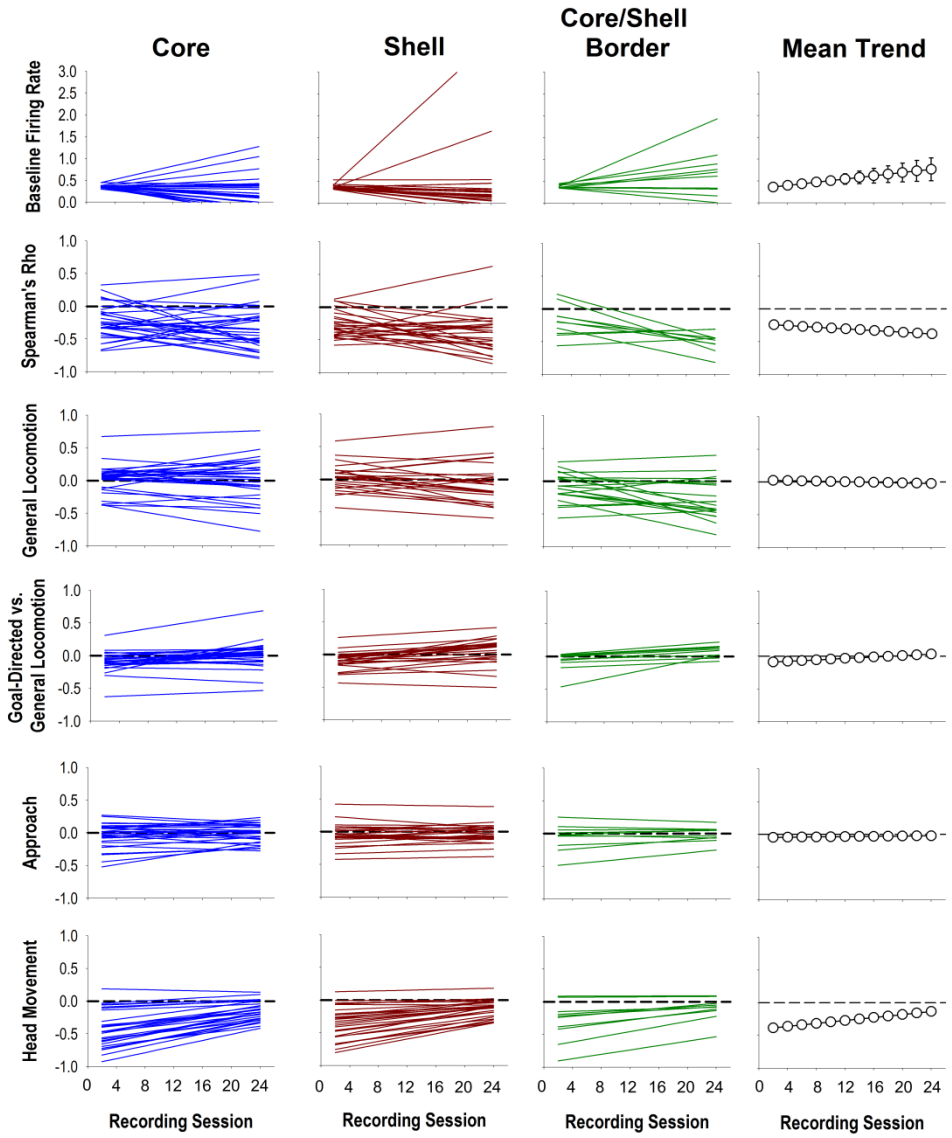


Figure 7. Empirical Bayesian estimates of linear fit for neurons localized to the Accumbens Core (blue), Shell (red) and Core/Shell border (green) as well as the mean fit for the observed trends in all subregions (right column). Individual rows represent neural events tracked over sessions. Baseline firing rate represents the raw firing frequency (spikes/second) of neurons. Spearman's Rho values represent the correlation between firing rate and drug level over sessions. General Locomotion, Approach, and Head Movement represent firing during each event when compared to baseline firing rates using the change ratio ΔFR_{event}=(Event - Baseline)/(Event + Baseline). Values for this ratio range between -1 and +1 with negative and positive values representing decreases or increases from baseline firing rate, respectively. Goal-Directed vs. General Locomotion portrays a similar change score for firing during approaches compared to firing during general locomotion [$\Delta FR_{\text{Goal-Directed locomotion}}$ =(Approach - General locomotion)/(Approach + General locomotion).

Figure 8

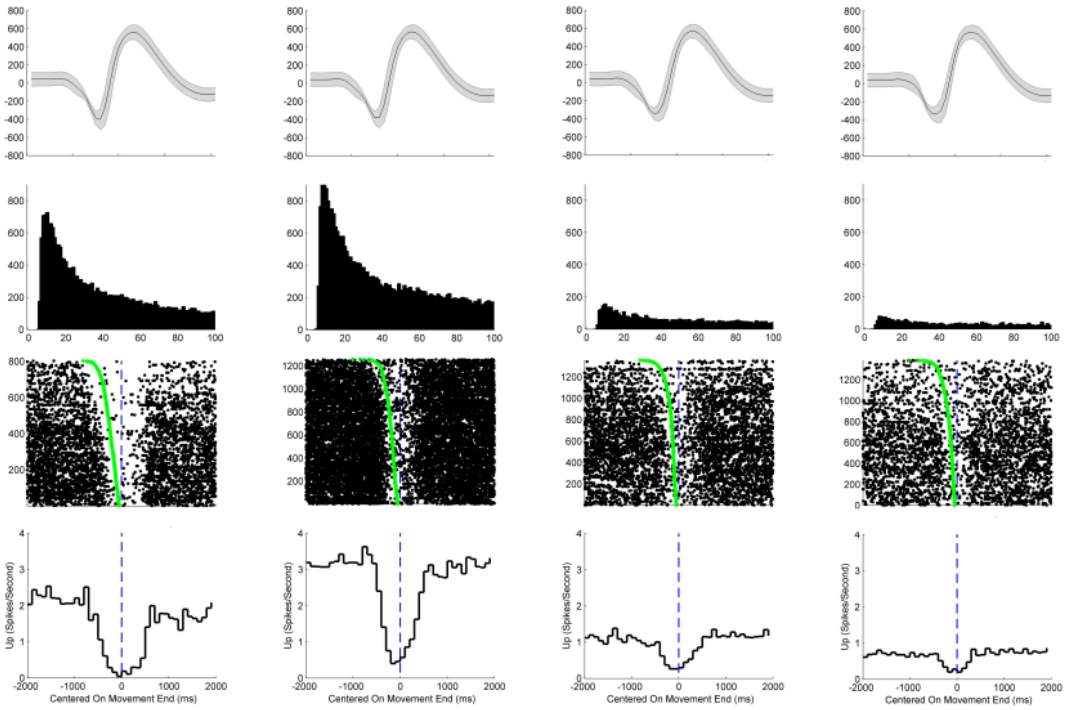
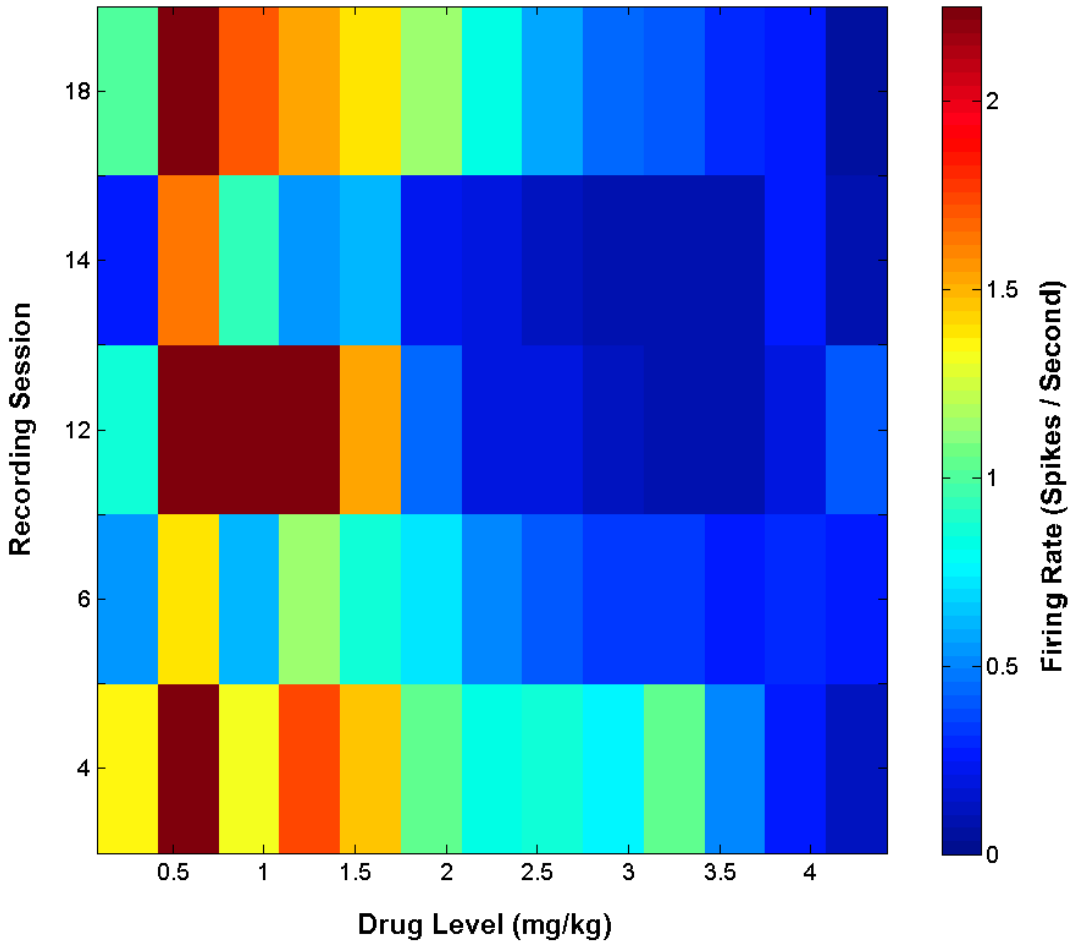


Figure 8. A single neuron exhibiting head-movement related firing over sessions. The top row shows the waveform of the neuron over recording sessions. The second row shows a histogram for the neuron's inter-spike interval (ISI) over sessions. Each histogram is free of spikes during the neuron's natural refractory period (~2ms). The third and bottom row show a raster and corresponding peri-event time histogram (PETH) centered on the end of each operant head movement. The green line shown in each raster represent the start of each head movement. Head movements are sorted for duration within the raster.

Figure 9



*F*igure 9. A representative neuron showing a relationship between firing rate and calculated drug level over sessions. Firing rates are highest at lower drug levels on all recording sessions and decrease as drug level increases. Individual recording sessions are shown on the Y-axis, calculated drug level is shown on the X-axis and firing rate is shown on the Z-axis (color scale).

APPENDIX A.

A 1.0- Histograms of Neural Firing Patterns.

Recording sessions were modeled as a continuous, linear variable in SAS PROC GLIMMIX. These data are represented as linear trends in Figure 7. As an extensions of Figure 7, histograms are shown across sessions for 1) Spearman's rho coefficients for the correlation of firing rate and drug level 2) change ratios for neural firing during locomotion relative to baseline firing rate 3) change ratios for approach related firing relative to locomotor related firing 4) approach related firing relative to baseline firing rates and 5) response (head-movement) related firing relative to baseline firing rates. Each histogram represents the distribution of correlation coefficients or change ratios on a recording by recording basis. Recording sessions represented blocks of two training sessions. Change ratios were calculated as: ΔFR_{event}=(Event - Baseline)/(Event + Baseline). Values for this ratio range between -1 and +1 with negative and positive values representing decreases or increases from baseline firing rate, respectively.

Figure A1

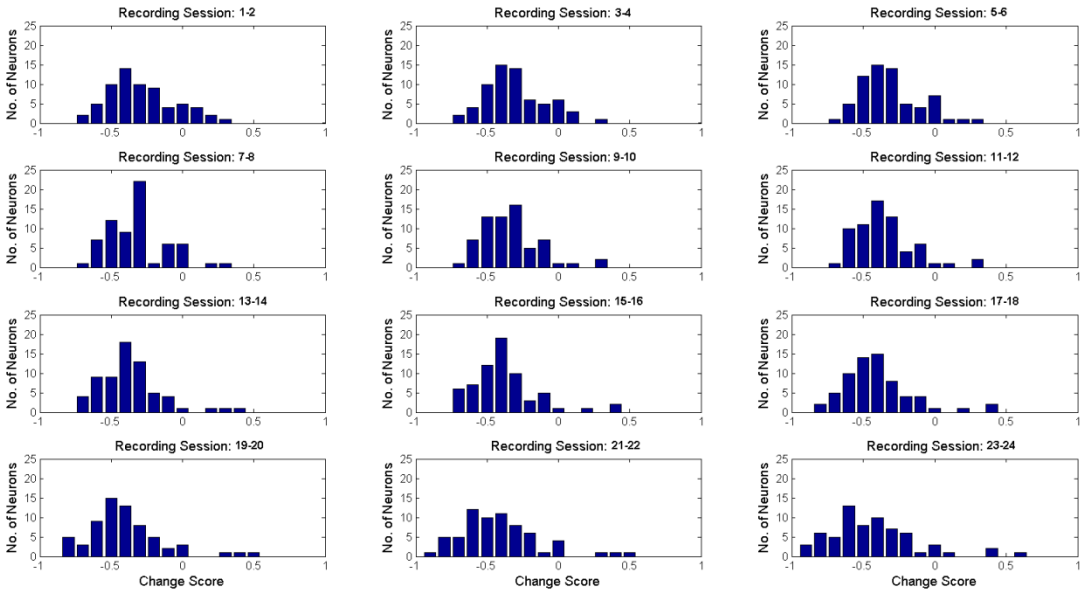


Figure A1. Histograms showing the distribution of Spearman's rho coefficients for the correlation of firing rate and drug level across recording sessions.

Figure A2

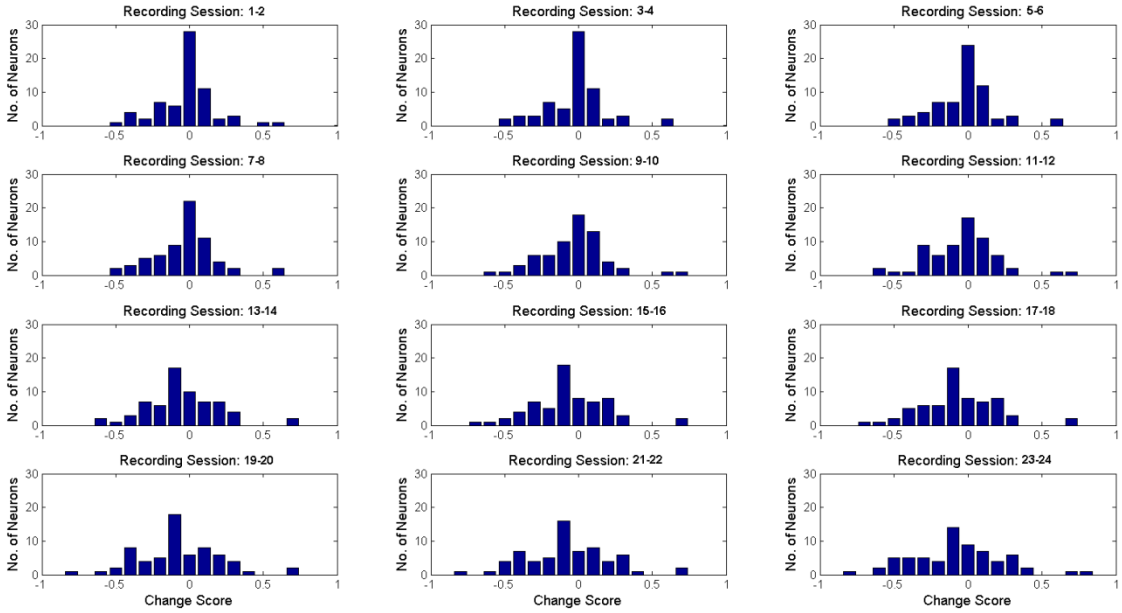


Figure A2. Histograms showing the distribution of change ratios for firing during general locomotion relative to baseline firing rates.

Figure A3

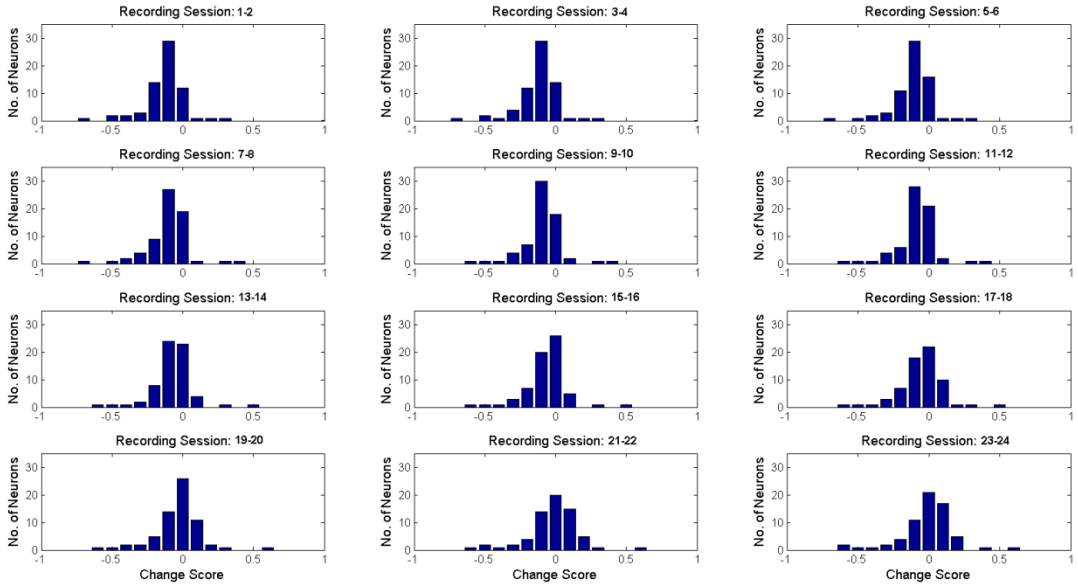


Figure A3. Histograms showing the distribution of change ratios for firing during approach to the operant relative to general locomotion firing rates.

Figure A4

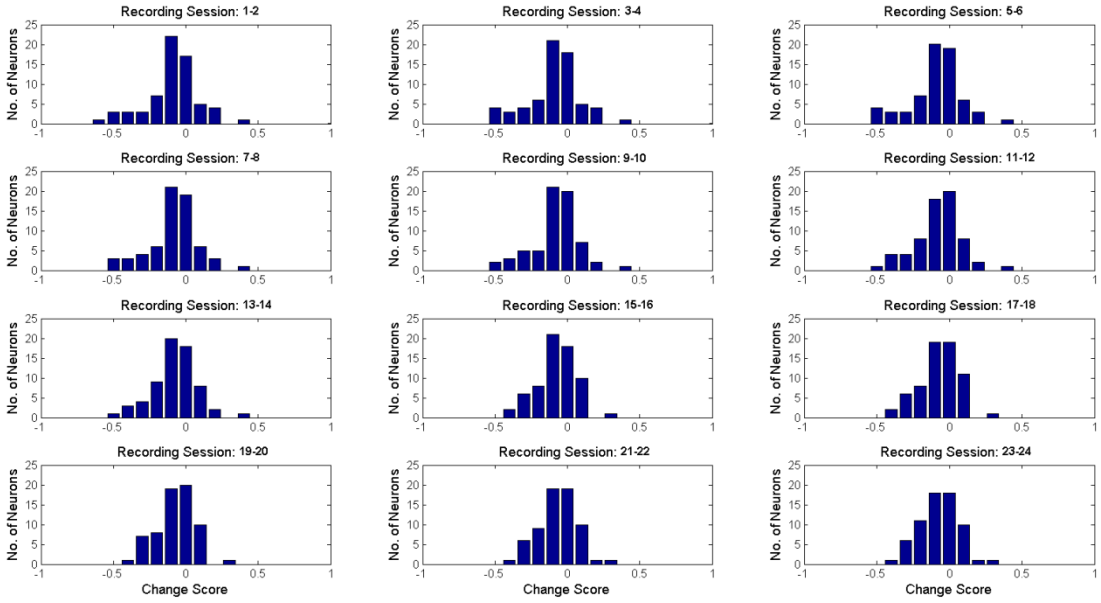


Figure A4. Histograms showing the distribution of change ratios for firing during approach to the operant relative to baseline firing rates.

Figure A5

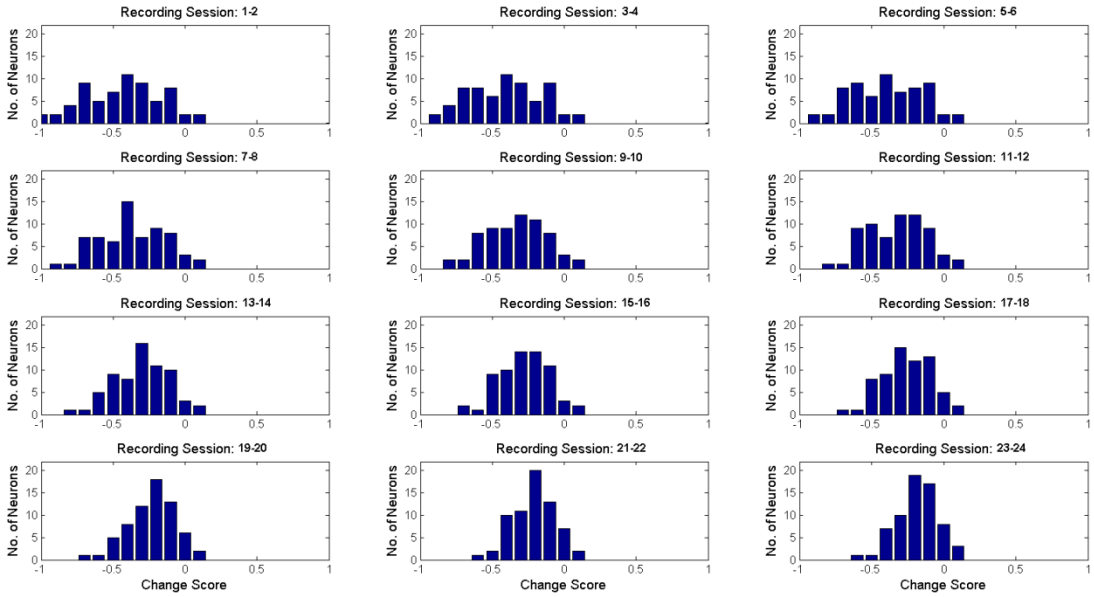


Figure A5. Histograms showing the distribution of change ratios for firing during operant responses (head movements) relative to baseline firing rates.