Heterogeneous development of drug abuse: Individual differences and predisposition to addiction.

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ABSTRACT OF THE THESIS

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The ability to identify individual predispositions to abuse drugs is important for long-term prevention of drug addiction. Drug addiction is in part driven by negative affect and is reinforced by compulsive resumption of drug taking. This problematic nature of addiction is thought to be influenced by drug-craving, triggered by reinvigoration of previous drug-associated environmental and contextual cues. The mesolimbic dopamine system is vital for the regulation of goal-oriented behaviors, which include drug, food, gambling, and sexual seeking. Impairment of the dopaminergic system dramatically influences addictive tendencies among individuals. Cocaine, and other drugs, “hijack” the reward system, and elevate dopamine in critical relay structures, such as the nucleus accumbens (Nac) which is a target of cue-associated addiction research because of its limbic-motor integration. Anatomically, the Nac is divided into core and shell subregions. Nac-core is “downstream” from the shell, is striatal-like, and projects to premotor areas which influence movement and goal-oriented behaviors through laterally spiraling striatal connections. The Nac shell is considered ‘upstream’ of the core and receives motivational input from the amygdala, hippocampus, and other limbic processing regions. Addiction researchers are

able to isolate differences in cue-predisposition through Pavlovian autoshaping (or STGT), which identifies two distinctive behavioral phenotypes; 1) Goal-trackers (GT), who approached the reward-port and 2) Sign-trackers (ST) who approached & attacked the lever-CS. ST have been theorized to incentivize reward cues and thereby prone to develop compulsive behavioral disorders such as addiction while GT animals have been largely ignored or used as a control to ST in addiction modeling. Notwithstanding historical focus, we found high intake GT abandon pre-drug tone-discrimination and compulsively respond in the absence of the cue, resulting in high rates of uncued maintenance drug seeking which drove higher drug intake. High intake, but not low intake, GT lacked the ability to control their internal drug level, which escalated to the highest recorded levels of any group during Hits. Non-GT titrated drug intake throughout the session, and DL did not significantly fluctuate between Hits and Misses within session. Additionally, high intake GT Nac core and shell neurons that were ‘silent’ during Misses became ‘active’ during Hits in the same session, suggesting Nac could influence motivation to seek large quantities of drug. No other group demonstrated these trends, and because addiction is known to impact people of all backgrounds, GT and Non-GT may be prone to develop two distinct formations of drug addiction based on inherent phenotype (goal vs cue oriented). Specifically, GT may represent a distinct animal model of addiction, one in which intense drug seeking is not highly influenced by contextual cues, but one where an internal ‘urge’ to take drugs results in an inability to control their drug intake.

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2. Introduction:

2.1 Drug Abuse and cues

Drug addiction is one of the greatest health concerns of the modern world. In addition to the devastating societal impacts, a recent troubling analysis of the true financial impact of addiction in America estimated costs of over 500 billion dollars in 2015 alone (The Council of Economic Advisers, 2017) and it is estimated that over 54,000 deaths were due directly to overdosing on illegal drugs in 2016 alone (Rudd et al., 2017).

Animal models of drug addiction suggest all subjects acquire drug self-administration (Coffey *et al.,* 2015), and individuals differ in their “preferred” drug level (Root 2009; Root et al, 2011). Traditionally known as titration, animals maintain their preferred satiety level, and stave off withdrawal, by responding when drug level falls below this level in a manner of negative reinforcement (Zimmer, 2013; Barker et al, 2014).

Human addicts often report a snowballing loss of control (Hammer *et al.,* 2012). Further, over time, these users were unable to obtain the same ‘pleasurable’ experience they once did, suggesting that long term usage involves negative rather than positive reinforcement (Baker *et al.,* 2004). One explanation for seemingly contradictory drug-seeking behavior is that cue-induced urges or craving for drugs becomes a powerful drug-seeking trigger, despite the myriad of negative consequences associated with previous drug dependence (Volkow *et al*., 2008). In fact, drug relapse has been theorized to be ‘cue-induced’ such that

these associations can trigger persistent drug seeking (Waters *et al*., 2014) even in the absence of drug itself (See *et al.,* 2003).

2.2 Predisposition to reward cues, sign vs goal-tracking

Researchers interested in determining predisposition to cue-induced relapse and drug seeking have utilized Pavlovian autoshaping as a tool for identifying individual differences in 'cue-reactivity' (Tomie, et al., 2008). Autoshaping is alternatively known as a sign vs goal-tracking (STGT). It is revealed by a classical conditioning procedure which involves the presentation of a conditioned stimulus (CS), such as a retractable lever, followed by an unconditioned stimulus (US) reward, such as a sucrose pellet. When animals bred for high or low locomotor performance in a novel environment were exposed to an autoshaping procedure, two distinct groups emerged. Bred-high locomotor animals approached and attacked the CS, and were defined as sign-trackers (ST), while bred-low locomotor animals ignored the CS (lever) and directly approached the reward (port). The bred-low locomotor animals were defined as goal-trackers (GT) (Robinson & Berridge, 1993; Flagel *et al.,* 2010). It has been hypothesized that ST animals were prone to incentive sensitization to the CS, whereby the actual cues that predicted rewards became salient and motivational (Robinson & Berridge, 1993; Robinson & Flagel 2009; Flagel *et al.,* 2010). Although STGT traits are hereditary, the two phenotypes ST and GT regularly occur in outbred animals as well (Robinson & Flagel, 2009).

Due to this heightened cue-reactivity, ST have historically been studied with regard to vulnerability to compulsive behavioral disorders, such as drug addiction

(Piazza *et al*, 2000; Flagel *et al.,* 2007; Robinson & Flagel, 2009; Flagel *et al.,* 2010; Saunders, Yager & Robinson, 2013). ST demonstrate a higher preference for choosing 'drug' over 'food' compared to GT (Tunstall & Kearns, 2015). Nonetheless, this same study showed no STGT difference in 'total cocaine reinforcements'. ST have also been shown to emit higher rates of 50 khz 'positive' calls when placed into an environment previously associated with cocaine (Meyer & Robinson, 2012) and show stronger seeking behavior during 'cocaine-cue' reinstatement (Yager & Robinson, 2015). ST and GT have been shown to ingest drugs at similar rates across days (Saunders & Robinson, 2010) and self-administer the same amount of drug at low doses (Bardo *et al*, 2006). Interestingly, removal of a CS signaling cocaine delivery resulted in reduction in cocaine infusions/minute in ST but not GT (Saunders & Robinson, 2010, 2013). GT also display consistent levels of focused stereotypy across sessions, while ST increase over the same time period (Flagel *et al.,* 2008). As sensitization is thought to be representative of salience of environmental cues (Kilbey & Ellinwood, 1977), GT may be more sensitive to incentivize cues immediately necessary for reward (such as the food-port during the pretest, or the operant nosepoke corner) while ST interact with local stimuli that are more obvious (eg, the CS-lever in the STGT pretest, or the tone-cue during cocaine SA) but may not be 100% of the necessary task. For example, GT display higher contextual renewal than ST when drug-seeking behavior was extinguished in a setting other than one paired with cocaine (Saunders & Janak, 2014).

2.3 Cues, Drug Addiction, and the Nucleus Accumbens

The influence of environmental or contextual cues can re-invigorate drug associations within motivational brain areas such as the mesolimbic dopamine system (Ikemoto & Wise, 2004; Thomas, Kalivas & Shaham 2008). The mesolimbic dopamine system has long been associated with regulation of goal-oriented behavior, such as food- or drug-seeking. Imbalances within the dopaminergic system dramatically influence addictive tendencies among individuals (Grimm *et al.,* 2003). Dopamine (DA) itself is heavily implicated in reward-based learning. Abuse of drugs (such as cocaine) appear to "hijack" this system by supranormally elevating concentrations of DA in key regions such as the nucleus accumbens (Nac) (Willuhn et al, 2014).

The Nac is a critical relay structure within the mesolimbic dopamine system, which is involved in forwarding limbic/motivational signals to premotor areas. The Nac's principal neurons, which comprise 95% of its neuronal population, are slow firing, medium spiny neurons (Kemp, Powell 1971). The Nac is divided into core and shell subregions. Anatomically, the core is "downstream" from the shell (Haber, 2000), and is striatal-like, with projections to premotor areas which influence movements through laterally spiraling striatal connections. The shell is considered 'upstream' and receives motivational input from regions such as the amygdala, hippocampus, and limbic cortical processing regions (Chesselet et al., 1998; Parkinson *et al.,* 1999, Pockros *et al.,* 2011). Processing of cues is a critical early stage of "limbic-motor integration" (Nauta et al, 1978) suggestive of laterally spiraling circuitry (Haber et al, 2000). Due to this motivational-striatal-

motor relationship, the Nac has been targeted frequently to explore the motivational mechanisms underlying drug-seeking behavior, addiction, and relapse (Ghitza *et al.,* 2003; 2004; Lin & Pratt 2014; Cui, Thakkar, Sullivan, *et al.,* 2015).

Acquisition of drug self-administration (SA) (Coffey *et al.,* 2015) and lengthy withdrawal from long-term use of cocaine are both associated with increased synaptic plasticity in the Nac core (Purgianto *et al*., 2013). Optogenetic *stimulation* of MSNs within the Nac has been shown to induce conditioned place preference in mice (Kravitz *et al*., 2012) while optical *inhibition* of Nac core and shell MSN neurons reduced place-preference in a cocaine-paired CPP experiment (Hikida *et al*., 2013). DA agonist injections into the Nac demonstrated the shell as the more effective portion of the accumbens in direct drug reinforcement (Carlezon et al., 1995; Carlezon & Wise,1996). Nac shell neurons also demonstrate strong and selective changes in firing in response to drug tone-cues but not to a neutral tone, while core neurons responded less strongly and nonselectively to both tones (Ghitza et al, 2003). Conversely, core but not shell neurons exhibited robust firing patterns correlated with the drug seeking approach/response, consistent with the core's striatal-like connections (Ghitza et al, 2004).

2.4 Objectives and goals

Sign-trackers have historically been the focus of addiction research, while GT counterparts have been used typically as a comparison group. With STGT phenotypes being translatable to humans (Garofalo & diPellegrino, 2015) and

drug addiction known to affect people of all backgrounds (Grant *et al.,* 2006), it is likely that individual differences among humans may enable heterogeneous neurobehavioral formations of addiction. In order to study these individual differences in addiction predisposition, this study sought to determine if GT represented a distinct model of addiction susceptibility, rather than simply a 'control' group. GT may represent a relatively ignored population in which subjects are not reactive to external or internal drug cues but whose exaggerated levels of drug seeking may nonetheless reflect tracking of their drug related goal. This study involved three main hypotheses based on literature review and data which demonstrated similar drug intake amongst STGT subpopulations:

Hypothesis 1: *Animals defined as GT will show no difference in drug intake from Non-GT*. Although an understudied subgroup, GT animals have been shown to ingest similar quantities of drug as non-GT animals (Saunders & Robinson, 2010) and thus we predict that average drug intake will be stable a function of a subject's initial pretest autoshaping results; i.e., individuals that were more GT-like in the pretest will show no difference in drug intake compared to those that show greater sign-tracking tendency, i.e., higher lever pressing (Non-GT) on a continuum of lever pressing. It is also predicted that GT and Non-GT will display similar rates of tone/task acquisition and similarly escalate cocaine intake across sessions, another key measure of addiction in animal models (Ahmed & Koob, 1998).

Hypothesis 2: High Intake *GT versus Non-GT animals will be sensitive to different types of cues, and will therefore self-administer drugs differently*.

Animals are known to differ in average preferred drug level (Root et al, 2009; Root et al, 2011) and preference for high drug level is known as a 'warning' sign in drug addiction (Wolffgramm & Hyene 1995). Addiction is not a 'one-size' fits all behavioral disorder, and it is likely that predisposition to external cues (ST) or goal-oriented cues (GT) could enable different 'severities' of addiction even if the overall drug intake is similar. Thus, we expect that within-session self-administration methods will differ in GT and Non-GT animals, where GT will make few uncued nosepokes and efficiently self-administer as much drug as they want. To better understand this phenomenon, analyses of hit/miss drug level during individual sessions were important to this study, specifically in GT and Non-GT animals that took large quantities of drug.

Hypothesis 3: *Shell firing in response to a drug predictive tone-cue*. Individual Nac core and shell neurons were recorded during cocaine self-administration sessions in order to identify firing responses to cues predictive of drug availability. The objective was to understand the underlying neural mechanisms on trials that evoked a 'Hit' vs trials resulting in a 'Miss', and restricted our planned *a priori* contrast comparisons to the same session, to ask: what was tone-evoked FR of the same neuron, on the same day, during trials on which drug was consumed (Hit) vs abstained from (Miss)? Based on this reasoning, previous research within our lab, and also literature implicating shell responsiveness to drug cues (Ghitza et al, 2003), we predicted strong shell responses in High intake GT and Non-GT animals during Hit trials and weaker responses of the same neurons during the same session's Miss trials.

Comparing FR differences on Hits vs Misses among high intake GT and Non-GT was predicted to reveal Nac processing of a cocaine cue at the instant during which the cue triggers a drug taking response. Due to our variable interval of drug availability in our experiment (1-6 minute variable timeout), the animal never knew exactly when the next infusion would be available. We expected both GT and Non-GT to show FR changes on Hits, when the animal was motivated to take drug. We also expected the same tone would not evoke firing changes on Miss trials (possibly because the drug is not 'as rewarding' or the animal is satiated on drugs). Therefore, during maintenance trials (during a binge), we expected Nac tone-evoked firing when the animal is 'motivated' to take cocaine on Hits. We also expected the same neurons would not change FR in response to the tone on Misses, Differences between Hits vs Misses could possibly reveal underlying spiking patterns triggered by cocaine use. Differences found in high intake animals would be valuable in light of the historic importance of Nac in drug addiction research.

Thus, any evidence supporting the aforementioned hypotheses would provide evidence for GT as an additional, yet distinctive, model of drug addiction.

3 Methods

3.1 Subjects:

Adult male Long Evans rats (Charles River, Raleigh, NC) were single housed for the duration of the STGT pretest and throughout the self-administration paradigm.

Procedure

3.2 STGT pre-testing paradigm

All rats (N=127) were initially characterized in a ST/GT paradigm to determine individual differences prior to the SA sessions over the course of 1 week. Consistent with Flagel *et al.* (2007), the animals were exposed to two days of pretraining. Each pretraining day, 50 pellets were dispensed on a 90s VI schedule over 25 minutes while the lever and light remained off. Following pretraining, the training schedule began and utilized a traditional autoshaping paradigm over five days: every trial during the training consisted of the presentation of a retractable lever and associated light cue (CS). After eight seconds a US-reward (banana pellet) was dispensed from the food port and the lever retracted into the slot. During the 8 second timeframe, all lever presses caused by pushing, gnawing, or manipulating the lever (i.e., number of lever presses) were recorded. Immediately after the lever retraction/reward delivery, a 30-90 second ITI began prior to the next trial. Each day consisted of 25 trials.

3.3 STGT Behavior Analysis

The lever presses observed during the final day of STGT pre-testing were analyzed to identify animals with strong GT traits. Lever pressing is viewed as a continuum in which more presses during the STGT pre-test are associated with sign-tracking, and fewer presses are associated with goal-tracking, i.e., tracking the reward port. A reasonable cutoff for total number of lever presses was sought to classify animals that showed strong goal-tracking behavior and thus were categorized as GT. The number of lever presses observed for each animal on the last day of STGT pretesting was graphed on a histogram and a cutpoint of 35 lever presses was determined to adequately demarcate goal-trackers for SA (n=8, GT) from Non-goal trackers (n=5, Non-GT)and was chosen through a combination of taking into account standard practice in the field of STGT, and was similar to observed GT lever presses as reported by a representative of the Robinson lab (personal communication, November, 2017) and visual inspection of our own STGT histogram (Figure 1). The chosen cutpoint of 35 represents the lowest 80th percentile of lever presses and isolates a subset of strong goal-trackers from the remaining population and also provides enough N for subsequent analyses.

Self-Administration Paradigm

3.4 Surgery:

Following the pretest, animals were selected based on STGT traits (N=8 GT, N=5 non-GT). Subjects were permanently implanted with an intravenous jugular

catheter, and a 2x8 stainless steel microwire array (MicroProbes for Life Science, Gaithersburg, MD) was then lowered into the right nucleus accumbens core and shell, according to the Paxinos & Watson *Rat Brain in Stereotaxic Coordinates* (1997) atlas in order to perform single unit recordings. For detailed surgical description, see Barker *et al.,* (2014). Following surgery, animals recovered for one week and remained in their SA experimental chambers 24/7 for the duration of SA.

3.5 Self-Administration Apparatus:

The clear Plexiglas experimental chambers included a corner with a customized 6-photocell device used to record head movements (Root *et al*, 2011). This device was fixed to the outside of the Plexiglas for the entirety of the experiment and recorded all responses (nosepokes) in the presence or absence of the tone-cue discriminative stimulus S^D. Post-operative animals remained in these experimental chambers for the duration of the experiment, and their weight was kept at 340±10g by compensation with food rations as necessary to prevent weight loss (e.g., due to elevated motor behavior).

3.6 Cocaine Self-Administration:

SA sessions consisted of cocaine SA for 6 hours/day, 7 days/week. Each session began at the light onset of the 12/12 light/dark cycle with the removal food/water and by 'plugging in' the animal to the recording harness every day for 14 days. Cocaine availability was signaled by the presentation of the 30 sec 3.5 kHz tone-cue, i.e., discriminative stimulus or S^D. A nose-poke response at any

time during the 30 sec tone halted the tone and activated the cocaine pump which distributed a 0.7 mg/kg/infusion of cocaine (0.24mg/0.2mL administered over 7.5s). The operant response was a nose-poke into any of the 6 infrared photocells in the "operant photocell corner." The corner was available at all times, to facilitate acquisition of the S^D properties of the tone; the tone was absent for 18 hours overnight, occurring only every 1-6 min during the 6-hour daily session. Thus, rats had the opportunity to learn that the tone was a perfect predictor of cocaine availability, contingent upon a response, but that responding in the absence of the tone, such as during overnight hours or during the intertrial interval, was never reinforced. The 1-6 min interval was designed so that the tone occurred during both satiety and sub-satiety drug levels (see below).

For purposes of behavioral analyses, a successful cocaine infusion elicited by a nose-poke response during the tone is defined as a reinforced response, or **"Hit,"** trial during that day's session. Each trial on which the tone did not evoke a hit is defined as a **"Miss,"** i.e., the animal "missed" the opportunity to self-infuse drug. Nose-pokes during the ITI did not deliver a cocaine infusion but were recorded as "uncued" drug seeking responses, or "**RB**."

Consistent with Coffey *et al.* (2015), the cocaine SA paradigm was subdivided into three distinct periods:

1) All trials leading to the first Hit were defined as the **"Pre-Drug"** period. Pre-Drug and Loadup periods (see below) at the start of each session used a 40 sec time-out instead of the 1-6 min interval, in which there was no tone presentation and all nose-pokes observed during the 40 sec time-out were recorded as

uncued responses. The rate of uncued responses was defined as “NoTone RB,” and was calculated as

$$NoTone\ RB = \left(\frac{Number\ of\ Uncued\ Responses}{Pre{-}Drug\ Total\ Summed\ ITI\ Time}\right) \quad (1)$$

where the denominator of Total Pre-Drug ITI Time is summed over multiple Pre-Drug 40 sec time-out periods. Conversely, the so-called rate of cued responses during Pre-Drug was defined “Tone RR” and was calculated as

$$Tone\ RR = \left(\frac{1}{Pre{-}Drug\ Total\ Summed\ Tone\ Time}\right) \quad (2)$$

where the 1 in the numerator refers to the first Hit of the session that demarcates the transition from Pre-Drug to drug, and enables the next session period (Loadup). The denominator of Total Pre-Drug Tone Time is summed over the total amount of time that the tone was on, and drug was available, leading up to the first Hit during the session.

2) The **“Loadup”** period consisted of each session’s first 10 Hits, and used identical operant conditioning parameters during tone and ITI periods as the Pre-Drug period.

3) The **“Maintenance”** period was defined as all trials following Loadup, i.e., 11 Hits and beyond. During Maintenance, a random number generator was used to present the tone S^D pseudo-randomly at variable intervals ranging between 1-6 min in order for the animal to experience being ‘above’ or ‘below’ satiety on 50% of the trials (see below: Drug Level Analysis). Traditionally, an animal seeks to maintain an individual ‘preferred’ DL (or satiety level) and will infuse when that

level is low in order to stave off withdrawal by responding when drug level falls, in a manner of negative reinforcement (Zimmer, Dobrin & Roberts, 2013). It is expected that an animal would seek an optimal DL, taking Hits when DL is low (and is below satiety) while ignoring opportunities to self-administer drug (Miss)when DL was above satiety (Root *et al.,* 2009, 2011).

The session ended at the 6 hour time limit, or 80 infusions (whichever arrived first). This study primarily focused on the Maintenance period, and thus any sessions which resulted in fewer than 10 total reinforced responses were not included in the analyses.

3.7 Histology:

Nac identification and separation of core and shell: Immediately following the final day of recording, the animal was injected with a lethal dose of sodium pentobarbital (150-200 mg/kg i.p.). In order to identify the exact location of all microwire tips, a 4 sec, 50mA current was applied to each microwire to leave trace amounts of iron (to be stained). The animal was then perfused with saline and 4% paraformaldehyde. The brain was removed and stored in the same concentration of paraformaldehyde for two days. Whole brains were transferred to a 30% sucrose-solution for several days and then sectioned (50 μm) through the Nac with an extra ~1.5 mm rostral and caudal to ensure each wire is located. Following slicing, tissue was mounted and organized anterior to posterior in order to track wires. Slices were stained with calbindin d-28k to allow for differentiation between core and shell. Iron deposits were then stained with a 5% potassium ferrocyanide and 10% HCL during the ‘washing’ process. Photographs of every

slice were taken and all 16 microwire tips were required to be accounted for. 'Missing wires' or wires located outside the Nac were discarded. A sample of the histology is shown in figure 2.

3.8 Electrophysiology During Cocaine SA:

Extracellular recordings: All neural signals were filtered through a customized preamplifier and then stored offline for analysis using specialized SciWorks spike-sorting software (Datawave Technologies, Longmont, CO) to isolate neural spikes from background noise. Such spikes were identified by parameters of their waveforms: peak time, peak voltage, and spike height were used to separate the single neuron activity.

Tracking the same neuron over days: Recording the same neuron over sessions was vital to this study. Standard practice is to isolate analysis of FR around a repeated event, such as the onset of the S^D tone-cue which signals cocaine availability. An example FR of a single neuron in response to the tone S^D, centered on the onset of the tone, is shown in Figure 3, illustrating a strengthening of that FR over sessions. Trials centered on the S^D were combined across sessions to average trial-to-trial variability and identify patterns of firing in response to the cue. Firing rates from days 4 (Pawlak *et al.,* 2010) to 15 (Peoples *et al.,* 1999) of SA were compared to early days 1 to 3 to assess differences related to learning the task. Interpretations were informed by the facts that MSNs are readily identified by waveform (Kulik et al, 2017) and our lab has demonstrated stability of single unit recording over sessions (Peoples *et al.,* 1999; Tang *et al.,* 2008; Coffey *et al.,* 2015) utilizing the following parameters: 1)

Waveform must be recorded from the same microwire; 2) Waveform and ISI histogram must be similar across days; 3) Neural discharge must not occur within the first 2 msec of the ISI histogram, i.e., evidence of a single neuron's natural refractory period (Kosobud, Harris & Chapin, 1994; Peoples *et al.,* 1999); 4) An uncommon event is the appearance of two different units on the same wire. If the second unit meets all criteria for a single neuron, and fires within the 2 msec window after neuron one's discharge, then it will represent a second individual neuron. Any recordings which failed to meet these criteria were discarded. Loss of stability was readily recognized, after which data recorded from that wire were discarded.

Analysis

3.9 Behavioral Analysis of Cocaine SA:

Initial behavioral analyses involved analyzing the relationships between the dependent variable of cocaine intake, the subject variable of STGT, and the experimental variables of Session and Tone, which signaled drug availability (Tone-on vs. Tone-off). Cocaine intake was tracked by observing reinforced responses ("Hits") during the SA session. All analyses were conducted using SAS software (SAS Institute Inc., 2002-2012).

Cocaine Intake by "Sign Tracking": Average subjects' total Hits (nosepoke during tone) during all late sessions in the SA paradigm were regressed on the number of total lever presses observed on the last day of pre-test sign tracking. Lower numbers of observed level presses are associated with strong goal-tracking

behavior. The analysis was conducted using PROC REG in which a linear regression relationship between average total hits and total lever presses was specified (figure 4a)

Cocaine Escalation among GT and Non-GT: Total number of Hits was regressed over Session (1 – 15) and on STGT (GT vs. Non-GT). The analysis was conducted using a linear mixed ANOVA model in PROC GLMMMIX in which total Hits was specified as the dependent variable, Subject was specified as a random effect, Session was specified a continuous independent variable, and STGT was a categorical independent variable. The interaction between session and STGT was also specified. The dependent variable was defined as Gaussian normal, and robust standard errors were specified. (Figure 5)

Tone Acquisition: In order to verify that animals acquired the operant nose-poke task and displayed tone acquisition, the rate of response (Response-Rate) during all Pre-Drug trials was analyzed. Response-Rate during tone (Tone-on, Cued Drug-Seeking, Equation 2) when drug was available, vs during the ITI (Tone-off, Uncued Drug Seeking, Equation 1) when drug was unavailable, were analyzed using a linear mixed ANOVA model in PROC GLIMMIX. Both rates were included in the dependent variable. Subject was specified as a random effect, and Sessions and Response-Rate (Cued vs. Uncued Drug-Seeking) were specified as independent variables. Sessions were categorized as “Early” (1-3) or “Late” (4-15). Uncued Drug-Seeking was calculated as total uncued drug seeking responses (RBs, unsuccessful nosepoke) divided by the total number of tone

presentations during maintenance, resulting in rate of uncued drug seeking responses per maintenance trial. (Figure 6).

Additionally, the interaction between Sessions and Response-Rate was included in the model for high intake (>55 hits) and low intake (<55 hits) groups separately due to our planned interest in comparing how high intake GT and Non-GT increased their drug intake (figure 6c). Additionally, we planned on extracting all 4 slopes for GT and Non-GT, high and Low to test for non-zero slopes (test against 0) and report log and antilog (base10) scales. We had 2 planned comparisons, high GT slope v high Non-GT slope and low GT v low Non-GT slope, and we created contrast codes to test if Rate of Uncued Drug seeking increased as a function of increasing %Hits during Maintenance. (Figure 6d) Because of skewness, the distribution of the dependent variable was specified as Gamma, with a logistic link function. Robust standard errors were specified. Follow-up simple effects F-tests were run only for factors present in significant main and/or interaction omnibus results.

3.10 **Drug Level During Cocaine SA**

Drug Level Calculation: The animal's estimated brain level of cocaine (drug level; DL) during each trial of the SA paradigm was a key factor that was necessary for various analyses. DL was calculated in real time at a resolution of 1 second, consistent with Pan *et al.* (1991). During each infusion, the animal's DL (uMole/L or uM) was computed using the following equation:

$$Drug\ Level = \left(\frac{d*k}{v(\alpha-\beta)}\right)\left(e^{-\beta*t} - e^{-\alpha*t}\right) \quad (3)$$

where d = dose of drug (mg/kg), k = 0.233 min (a rate of flow between two compartments), v = 0.15 L/kg (brain volume), α = 0.642 min & β = 0.097 min (constants that represent the redistribution and elimination of cocaine), and t = time (minutes) since the last infusion. Equation 3 is consistent with Lau & Sun (2002).

Average Drug Level as a function of STGT: Average subjects' drug level for all late sessions (4-14) in the SA paradigm were regressed onto the number of total lever presses observed on the last day of pre-test sign tracking. The analysis was conducted using PROC REG in which a linear regression relationship between average total hits and total lever presses was specified (figure 4b)

Drug Level as a function of Response, STGT, & Intake

For purposes of analysis, it was necessary to separate those sessions during which animals preferred higher intake from lower intake of cocaine. Approximately 50% of the animals preferred >55 infusions on average, which allowed us to categorize days as high (High Total Hits, high intake) or low (Low Total Hits, low intake) using 55 Hits as a cutpoint. This allowed us to make within group comparisons of GT and Non-GT animals that were prone to more severe aspects of drug addiction (high drug intake animals). It should be noted that this cutpoint was not made to compare across high and low intake categories, but rather to independently evaluate GT high intake vs Non-GT high intake separately from GT/Non-GT low drug takers.

DL values, as defined by Equation 3, were computed at each Maintenance trial and analyzed as a function of Response (Hit vs. Miss), STGT (GT vs. Non-GT), and observed Intake (High vs. Low) using a linear mixed ANOVA model, which was run using PROC GLIMMIX. Subject was specified as a random effect, all independent variables were specified as categorical, and all possible two-way and three-way interactions were specified. The dependent variable was defined as Gaussian normal, and robust standard errors were specified. As discussed above, only follow-up simple effects F-test comparisons that involve Response (Hits vs. Misses) were of primary interest. Therefore, the only post-hoc comparisons that were performed compared Hits vs. Misses for all four possible combinations of STGT and Intake , i.e., GT High, GT Low, Non-GT High & Non-GT Low intake as an analysis of drug level variability on Hits vs Misses nested within the same subject and session. (Figure 7a).

Due to our interest in self-administration pattern differences as a function of increasing drug intake, DL standardized change (SC) as a function of Total Hits across STGT categories was analyzed using linear mixed ANOVA to address the Hit/Miss directionality of the drug level fluctuations (seen in figure 7a). Drug Level SC between Hits and Misses was the DV (negative values indicating higher Miss DL relative to Hit DL within session), Total Hits was a continuous independent variable IV (covariate), and STGT was a categorical IV (figure 7b). Additional analysis of Self-administration patterns amongst the GT and Non-GT, were assessed at three levels of intake on the continuum of Total Hits (lowest = 10,

medium = 45, highest = 80), as they captured the full range of the continuous IV at the lowest, median, and highest drug intake thresholds (figure 7b).

3.11 Neural Activity During Cocaine SA

Neural Analysis: In order to study processing related specifically to the cocaine S^D, all post-tone (tone-evoked) changes in FR of each Nac neuron were compared to that neuron's baseline FR. FR for each trial was calculated as the number of discharges in a 200 msec window, starting at tone onset and ending 200 msec later, which corresponds to the earliest onset of tone-evoked movement). Baseline FR for each trial was FR immediately before tone onset, for a period of 200 msec, which was equal to that trial's post-tone time window of 200 msec.

This decisive cue processing is the critical early stage of "limbic-motor integration" (Nauta *et al,* 1978) suggestive of laterally spiraling circuitry (Haber *et al,* 2000). Neurons were recorded every odd day (1-15) to study firing rates of Nac core and shell neurons in response to the S^D on **hits vs misses** during the maintenance segment of the experiment (all trials following the 10^{th} Hit of that session).

Computation of "Neuron-sessions": Average Baseline and Post-Tone FRs across each individual session's trials were computed separately for Hit and Miss trials, which resulted in average FRs for "neuron-sessions" which were the main unit of observation for subsequent analyses. All analyses were nested within neuron. Computed Post-Tone neuron-session FRs were plotted as a function of Baseline

neuron-session FRs separately for the subgroups defined by all possible combinations of Response, STGT, Total Hits and Region (Shell/Core), and the Pearson correlation coefficient (*r*) for each subgroup was computed. In this case, the Pearson correlation served as a measure of "stability" of average neuron-session FR from Baseline to Post-Tone. High stability conditions are those in which the correlation is closer to +1, i.e., the neuron's average FR does not change greatly from Baseline to Post-Tone. In contrast, low stability conditions are those in which the correlation is closer to 0, i.e., the neuron's average FR changes significantly from Baseline to Post-Tone. Significance tests for each *r* value were computed. (Figure 8ab)

Zero-inflation analysis: It was observed in preliminary analyses that there were a significant subset of neuron-sessions in which a neuron was completely silent, i.e., exhibited an average FR = 0 during the 400 msec around the tone. Therefore, a two-stage approach for analysis was adopted that is standard for modeling so-called "zero inflated" data (Long, 1997; Zeileis, et al., 2008). The first stage involved dichotomously recoding average neuron-session FR in which 0 FR becomes 0 and non-0 FR becomes 1. The recoded dichotomous outcome variable is then analyzed using a logistic regression model. The second stage involves taking only the non-0 average FR values as the outcome variable and analyzing it by specifying a statistical model with a gamma distribution, with a log link, for the dependent variable. Additionally, this analysis was independently analyzed for baseline and post-tone, variable defined as Tone. All logistic analyses were first assessed to see if Tone was significantly different, and

collapsing baseline and post firing was only considered if this was the case. Collapsing across tone allowed a 400ms window to identify neural ‘silence’ or ‘activity’ for that trial, and averaged over an entire session for a ‘neuron-session.’ (Figure 9ab).

Logistic regression, core and shell: The logistic regression for the dichotomously recoded average FR values was run as a nonlinear mixed ANOVA model using PROC GLIMMIX, with neuron specified as the random effect, and Tone (Baseline vs. Post-Tone), Response (Hit vs. Miss), STGT (GT vs. Non-GT, and Intake (High vs. Low) as independent variables. All possible main and interaction effects were specified. Separate models were run for Core and Shell neurons. Robust standard errors were specified. As discussed above, only follow-up simple effects F-test comparisons that involve Response (Hits vs. Misses) were of primary interest. Therefore, the only post-hoc comparisons that were performed compared Hits vs. Misses for all possible combinations of STGT and Intake, i.e., GT-High, GT-Low, Non-GT-High, & Non-GT-Low.

Nonzero regression core and shell: The statistical model for the non-0 average FR values was run as a nonlinear mixed ANOVA using PROC GLIMMIX, with the dependent variable specified as a gamma distribution with a log link, neuron specified as the random effect, and Tone, Response, STGT, and Intake as independent variables. All possible main and interaction effects were specified. Separate models were run for Core and Shell neurons. Robust standard errors were specified. As discussed above, only follow-up simple effects F-test comparisons that involve Response (Hits vs. Misses) were of primary interest.

Therefore, the only post-hoc comparisons that were performed compared Hits vs. Misses for all four possible combinations of STGT and Intake, i.e., GT-High, GT-Low, NonGT-High, & NonGT-Low.

4 Results

Behavioral Results

4.1 Intake as a function of pretest "Sign-Tracking"

In order to identify differences in the overall average intake (Average total hits) among subjects on their individual pretest lever pressing (or degree of sign-tracking) (Figure 4a), a regression analysis was performed on each animal's average Hits as a function of pretest lever pressing results. The analysis revealed that the intercept ($a = 53.53$) was significantly different from 0, $t(1) = 7.55$, $p < .0001$. This intercept provided an additional justification for our separation of high intake (>55 Hits) vs low intake sessions (but not to directly compare High vs Low). Additionally, the slope for STGT Lever Press ($b = 0.098$) was not significantly different from 0, $t(1) = 0.50$, $p > .10$. A similar analysis of average Drug Level as a function of lever pressing (figure 4b) revealed similar results as slope for STGT Lever Press ($b = -0.01$) was also not significantly different from 0, $t(1) = -0.42$, $p > .10$. These results demonstrate that there was no increase observed in cocaine intake as a function of the individual animal's pretest lever presses, or individual "Sign-tracking" trait.

4.2 Acquisition of cocaine self-administration: increased cocaine intake over days

Although animals demonstrated stable drug intake as a function of increased pretest lever presses, we wanted to identify if animals defined as GT vs Non-GT would escalate their cocaine intake over sessions at similar rates. A linear mixed ANOVA model showed a significant main effect of Session, $F(1, 113) = 10.56$, $p = .0015$, which suggests escalation of cocaine intake, but not a significant main effect of STGT, $F(1, 113) = 0.53$, p. > .10, nor a significant interaction of Session by STGT, $F(1, 113) = 1.52$, $p > .10$. This analysis revealed that both populations acquired cocaine self-administration at similar rates (Figure 5), but it did not address whether this escalation of drug intake was due to additional selective tone-responses, or due to compulsive uncued drug-seeking in general.

4.3 Tone-acquisition: Response-Rate during Pre-Drug Trials

As both GT and Non-GT escalated cocaine intake at similar rates, we wanted to identify if this was due to more selective tone-responding. During daily pre-drug trials, Response-Rate during tone (Tone-on drug available, i.e., “Cued” drug-seeking) vs Response-Rate during the inter trial interval (Tone-off, drug unavailable, i.e., “Uncued” drug seeking) were compared in GT and Non-GT to assess acquisition of selective tone-discrimination. A linear mixed ANOVA model found significant main effects for Session (Early v Late) , $F(1, 327) = 6.06$, $p = .014$, and Response-Rate (Tone v ITI), $F(1, 327) = 6.40$, $p = .012$. , but not for STGT (GT v Non-GT) $F(1, 327) = 1.35$, $p > .10$. Significant two-way interactions were found for Session by Response-Rate $F(1, 327) = 6.41$, $p = .012$, but not for

Session by STGT, F(1, 327) = .66 p >.10, nor for Response-Rate by STGT F(1, 327) = .00 p >.10. The three-way interaction of Session by Response-Rate by STGT was also not significant, F(1, 327) = .12 p >.10.

Due to the significant two-way interaction of Session by Response-Rate, follow up simple effects were carried out on the effects of Response-Rate differences for each level of Session, and each level of Response-Rate. A significant effect of Response-Rate was found for Late Sessions, F(1, 327) = 27.63, p < .0001, but not for Early Sessions, F(1, 327) = 0.04, p. > .10. A significant effect of Session was found for Tone-on, F(1, 327) = 13.09, p = .0003, but not for Tone-off F(1, 327) = 0.01, p > .10. Thus, during daily pre-drug trials, both GT and Non-GT demonstrated evidence of tone discrimination, in that their response rate during the tone increased across sessions while uncued drug-seeking did not increase across sessions (figure 6a).

4.4 Tone-acquisition: Response-Rate during Maintenance (drug onboard)

Although both groups exhibited tone discrimination during pre-drug trials, the question remained whether selective responding during the tone cue *per se* influenced drug taking for the entirety of the session, or whether non-selective uncued drug seeking was the major factor in driving up session drug level. In fact, drug level was significantly correlated with Uncued Drug-Seeking in GT r=.76 and in Non-GT r=.65 (Figure 6b) but further ANOVAs were run to assess if uncued drug seeking differed amongst high and low drug intake for GT and Non-GT. For GT animals, Uncued Drug-Seeking was significantly greater on high than on low intake F(1,75) =14.21, p=.0003, and. Conversely, Non-GT did not exhibit

different rates of uncued drug seeking between high and low intake, $F(1,44)=1.32$, $p>.10$.

The same analysis was conducted for the subset of animals that contributed sessions to both high and low intake (i.e., animals who exhibited some sessions of >55 hits and other sessions of <55 hits). Similar results were found as GT animals that contributed to both high and low intake sessions had higher rates of Uncued drug seeking on high vs low intake $F(1,45)=1.32$, $p=.0043$, while Non-GT uncued drug seeking was not different among animals that contributed to both sessions $F(1,27)=1.01$, $p>.10$ (figure 6c). Thus, although uncued drug-seeking showed positive slopes as a function of drug intake (figure 6b), these findings suggest that compulsive and uncued drug seeking was the major factor in elevating GT drug intake, while Non-GT selectively increased their tone-responses (Hits).

An additional analysis of uncued drug seeking targeted slope differences as a continuum of drug intake from lowest to the highest intake (80 Self-Infusions, 100% Hits). We first extracted individual regression slopes for each of the subcategories (GT and Non-GT, high and low intake), and found that all log-adjusted slopes were significantly different from 0 for all subgroups: GT-High, $B = 4.67$, $t(112) = 12.22$, $p < .0001$, GT-Low, $B = 3.41$, $t(112) = 5.98$, $p. < .0001$, Non-GT-High, $B = 2.65$, $t(112) = 3.06$, $p = .0028$, Non-GT-Low, $B = 4.33$, $t(112) = 3.71$, $p. =.0003$. We then tested if these log adjusted slopes were different among the four subcategories using a log-linked generalized linear mixed ANOVA model. Significant main effects were found for %Hit, $F(1, 112) = 33.87$, $p < .0001$,

but not for Intake, $F(1, 112) = 0.56$, $p > .10$, or for STGT $F(1, 112) = 1.71$, $p > .10$. Significant two-way interactions were found for STGT by Intake $F(1, 112) = 4.19$, $p > .10$, but not for %Hit by STGT, $F(1, 112) = 1.17$ $p > .10$, or for %Hit by Intake $F(1, 112) = 0.86$ $p > .10$. A three-way interaction of %Hit by STGT by Intake was found to be significant, $F(1, 112) = 5.71$ $p = .0185$, which suggested that the slopes of uncued drug seeking were not identical at different levels of intake among GT and Non-GT. Post-hoc analyses were conducted on the slopes for the regressions of Uncued Drug seeking on %Hit for each of the subgroups of STGT and Intake to determine if uncued seeking differed at the different extremes of intake. Post-hoc comparisons between the GT vs. Non-GT High Intake showed a significant difference, $t(112) = 2.13$, p. = .035, but not between GT vs. Non-GT Low Intake, $t(112) = -0.71$, $p > .10$.

Interestingly, GT and Non-GT low intake showed similar slopes of uncued drug seeking (figure 6d), but differences were identified in high intake animals. Despite demonstrating acquisition of tone discrimination during pre-drug trials (Figure 6a) GT abandoned this selective tone-responding as soon as drug was on-board during maintenance, regardless of drug availability signaled by the tone (figure 6cd). That is, their drug intake was driven upward not by more selectively responding during the tone, but by a significant increase in overall responding.

Although Non-GT drug intake increased with uncued drug seeking (figure 6b), Non-GT high intake was not simply driven by non-selective responding, instead, their increased drug use was driven by more selective tone-responses. Non-GT exhibited pre-drug one-discrimination (figure 6a) and continued throughout the

Maintenance session evidenced by similar rates of uncued Maintenance drug seeking in High intake Low intake Non-GT (figure 6c) and similar slope of uncued drug seeking to low intake GT and Non-GT, which was a lower slope than high intake GT (figure 6d).

Therefore, Non-GT additional intake was driven by more selective tone-responses, while GT additional intake was driven by non-selective increases in both cued and uncued drug seeking. Animals compulsively respond when they are sub-satiety (Barker et. al., 2014), thus analysis of Drug Level within session was necessary to test whether GT DLs were routinely below satiety (and also whether they exhibited high miss DL relative to hit DL).

Drug Level Results

4.5 Session DL on Hits vs Misses: The population of GT and Non-GT exhibited similar rates of cocaine intake (figure 4ab), cocaine escalation (figure 5), and tone acquisition (figure 6a). However, GT demonstrated a greater overall response rate, regardless of drug availability signaled by the tone on high vs. low intake sessions, whereas Non-GT appeared to be driven by higher rates of selective tone-discrimination (figure 6cd), suggesting that the patterns of self-administration may have differed in animals that preferred higher drug intake. Preference for high drug level is known as a ‘warning’ sign in drug addiction (Wolffgramm & Hyene 1995). Although high intake animals appeared at similar rates in both GT and Non-GT (table 2), the fluctuations of within session drug level during individual sessions needed to be examined to test whether GT were unable to control their drug intake

Analysis of drug level (DL) differences between Hits and Misses utilized a linear mixed ANOVA model, and found significant main effects for Response, $F(1, 10586) = 4.33$, $p = .038$ and Total Hits, $F(1, 10586) = 79.19$, $p < .0001$, but not STGT , $F(1, 10586) = 0.00$, $p > 0.10$. Significant two-way interactions were found for Response by STGT, $F(1, 10586) = 3.70$, $p = .05$, Response by Total Hit, $F(1, 10586) = 18.22$, $p < .0001$, and STGT by Intake $F(1, 10586) = 13.65$, $p = .0002$. The three-way interaction of Response by STGT by Intake was significant, $F(1, 10586) = 4.16$, $p = .041$. Follow up simple effects tests were conducted for the differences between Hit vs. Miss (Response), for all subgroups defined by all possible combinations of STGT and Intake. The difference in drug level between Hit vs. Miss was found to be significant only for the subgroup of GT-High Intake, $F(1, 10586) = 15.85$, $p. < .0001$. None of the other subgroups were statistically different; GT-Low Intake, $F(1, 10586) = 0.15$, $p. > .10$, Non-Gt-High Intake, $F(1, 10586) = 0.95$, $p > .10$ Non-GT-Low Intake, $F(1, 10586) = 0.55$, $p > .10$. Thus, High intake GT exhibited significantly more variable DL on Hits vs Misses during the same session, suggesting they were unable to control their drug intake. In contrast, high intake Non-GT (and all low intake animals) appeared to restrict drug level fluctuations and were more ‘in control’ of their internal drug level during the session.

To address the directionality of the DL differences in high intake GT, and to confirm that DL was actually low on Hits and high on Misses in the other groups, DL standardized change (SC) were computed for each session and were then plotted as a function of the Hits for that session across STGT (figure 7b). A linear

mixed ANOVA was run in which Drug Level SC between Hits and Misses was the DV, Total Hits was a continuous IV (covariate), and STGT was a categorical IV. The ANOVA revealed significant main effects of STGT $F(1, 109) = 5.92$, $p = .0166$, and Total Hits $F(1, 109) = 4.48$, $p = .0366$ and a significant interaction was found for Total Hits by STGT $F(1, 109) = 10.49$, $p = .0016$. This indicated a differential relationship between Drug Level SC and Total Hits for the GT and Non-GT groups.

Inspection of Figure 7b indicated that the interaction between Total Hits and STGT was disordinal. That is, Non-GT showed a positive relationship of Drug Level SC indicating that as Total Hits increased so did the MissDL/HitDL relationship (consistent with 'titration'). In contrast, GT showed a negative slope, indicating that as Total Hits increased the Miss/Hit relationship inverted (inconsistent with 'titration'). Due to our interest in self-administration patterns amongst the highest GT and Non-GT, three levels of intake on the continuum of Total Hits were specified as it captured the full range of the continuous IV at the lowest, median, and highest drug intake thresholds (lowest = 10, medium = 45, highest = 80). Results indicated that for the lowest level of Intake, GT had higher Drug level SC for Misses, $t(109) = 2.23$, $p = 0.028$ and analysis of moderate intake showed no difference in GT and Non-GT Drug Level SC, $t(109) = .55$, $p > .10$. In contrast, analysis of the highest intake revealed that GT showed a significantly lower (and negative) DL change, while Non-GT showed a positive change $t(109) = -2.18$, $p = 0.0318$. This result is unexpected from the standpoint of substantial literature demonstrating titration (when GT are not analyzed

separately). It indicates that as a function of increasing Total hits (intake) GT had a higher DL on Miss that Hit, while Non-GT showed an inverse pattern consistent with titration (lower DL on Miss that Hit).

These findings suggest that despite similar overall drug consumption during high intake days (intake≥ 55 Hits), and similar rates of “high intake” sessions (table 2), analysis of within-session drug level differences revealed a key difference between High intake GT vs High intake Non-GT and all low intake animals. Specifically, as drug intake increased, the relationship between Miss/Hit became more positive in Non-GT and low intake animals, consistent with the concept of ‘titration’. Hit when their DL was low and Missed when their DL was high in an expected way typical of titration. Conversely, as intake increased in GT, drug level SC became significantly more negative, opposite of the expected ‘titration’. Peculiarly, GT animals had a higher DL on Hits than on Misses during sessions of high intake.

4.6 Neural Results

Our Neural analysis for both core and shell addressed of the following questions: What were the differences in tone-evoked activity (Baseline vs Posttone) in GT and Non-GT animals on Hits versus misses on the same day? Was there evidence of ‘silent’ or ‘active’ patterns on Hits vs Misses (logistic)? Finally, what was the distribution of FR in neurons that were only ‘active’?

4.6a: Nac Core neuron-session analysis: A logistic mixed ANOVA was used to model Core neuron-sessions where significant main effects were found only for

Tone (baseline vs posttone), F(1, 447) = 8.11, p = .0046 which signaled that collapsing baseline and post was not acceptable. Neither main effects of Response (Hit vs Miss), F(1, 447) = 1.54, p >.10, nor STGT , F(1, 447) = 1.07, p >.10, were significant. Significant two-way interactions were not found for any interactions of the factors: Tone by Response, F(1,447)= .41, p >.10, Tone by STGT, F(1,447)= 2.27, p>.10, Response by STGT, F(1,447)= 2.35, p>.10, Tone by Intake F(1,447)= 2.23, p >.10, Response by Intake, F(1,447)= 3.33, p>,05, STGT by Intake, F(1,447)= .35, p >.10 The three-way interaction of Tone by STGT by Intake was significant, F(1, 447) = 7.75, p = .0056. Nonsignificant results were found for other three-way interactions: Tone by Response by STGT, F(1, 447) = .09, p>.10, Tone by Response by Intake, F(1, 447) = .82, p>.10, and Response STGT by Intake, F(1, 447) = 1.36, p>.10. The four-way interaction was not significant, i.e., Tone by Response by STGT by Intake, F(1, 447) = 0.00, p>.10.

Follow up simple effects F-tests were run to analyze the differences between Tone (Baseline vs. Posttone) for all possible combinations of STGT and Intake. The difference between Baseline and Posttone was significant for the Non-GT High Intake group, F(1, 447) = 8.23, p. = .004 and for the GT low Intake group F(1, 447) = 4.39, p. =.037. The differences between Tone vs Baseline for all other subgroups were not significant, GT-High, F(1, 447) = 0.00, p. > .10 and Non GT-Low F(1, 447) = 0.00, p. > .10. Thus, Core neurons in High Non-GT (and low GT) showed evidence of tone-evoked activity on Hits but not on Misses (figure 8a).

Because a difference in tone-evoked Nac core activity was found, a follow-up analysis of all 'active' core neurons utilized a gamma mixed ANOVA model to analyze the continuous distribution; i.e., average neuron FRs pre and post tone. This gamma model found no significant main effects of Tone $F(1,265)=1.40$, $p>.10$ Response, $F(1, 265) = 1.20$, $p>.05$ or STGT, $F(1, 265) = 1.27$, $p>.10$. Significant two-way interactions were found only for Response by Intake, $F(1, 265) = 6.19$, $p=.013$ but not for Tone by Response, $F(1, 265) = 0.87$, $p>.10$, Tone by STGT, $F(1, 265) = 1.54$, $p>.10$, Response by STGT, $F(1, 265) = .08$, $p>.10$, Tone by Intake, $F(1, 265) = 1.61$, $p>.10$, or STGT by Intake, $F(1, 265) = 1.15$, $p>.10$. There were no significant three-way interactions; Tone by STGT by Intake, $F(1, 265) = 1.19$, $p>.10$, Tone by Response by STGT, $F(1, 265) = 0.16$, $p>.10$, Tone by Response by Intake, $F(1, 265) = 2.18$, $p>.10$ and Response STGT by Intake, $F(1, 265) = 0.20$, $p>.10$ were all nonsignificant. The four-way interaction was also not significant, where Tone by Response by STGT by Intake, $F(1, 265) = 0.94$, $p>.10$.

The two way interaction of Response (Hit vs Miss) by Intake (High vs Low) was significant, and follow-up simple effects F-tests were conducted to analyze logistic FR differences of Response (Hit vs. Miss) for all possible combinations of STGT (GT vs. Non-GT and Intake (High vs. Low). The distribution of 'Silent' (0 FR average for a session) vs 'Active' (Non-0 average session firing rate) revealed significantly more 'Active' neurons for Hit trials than Miss trials, but only for the GT-High Intake group, $F(1, 447) = 5.24$, $p. = .0226$. High intake Non-GT animals (and all low intake animals) showed similar distributions of logistic firing

on hits and misses: Non-GT High, F(1, 447) = 0.03, p. > .10, Non-GT-Low intake F(1, 447) = .78, p. > .10, GT-Low Intake, F(1, 447) = 1.33, p. > .10. Thus, underlying 'Activity' was different on Hits vs Misses: neurons that were silent on misses became active on hits in GT high intake, while no other group had neurons with similar logistic patterns (Figure 9a).

Therefore, in core neurons, evidence of tone-evoked activity was found on Hits (but not Misses) for High Non-GT and low GT (figure 8a). Also, high intake GT core neurons were 'silent' on Misses but became 'active' on Hits during the same session (figure 9a).

4.6b: Nac Shell: models of zero inflation, a binary analysis

The same logistic analysis (0 vs Non-0 FR) was then performed for Nac shell FR and compared the same neuron's 'silence' or 'activity' during the same recording session. An analysis of logistic FR patterns (0 vs 1) on tone (Baseline v Posttone) utilized a mixed ANOVA to identify the distribution of the 0 and Non-0 patterns on Baseline FR (-200ms) versus Posttone FR (+200ms). The binary analysis of Nac-shell activity (active vs silent) revealed significant main effects for STGT, $F(1, 544) = 5.19$, $p=.023$ but not for Tone, $F(1,544)=0.01$, $p>.10$ or Response, $F(1, 544) = 2.98$, $p>.05$. Significant two-way interactions were found for Response by STGT, $F(1, 544) = 9.37$, $p=.002$ and Response by Intake, $F(1, 544) = 8.48$, $p=.004$ but not for Tone by Response, $F(1, 544) = 0.68$, $p>.10$, Tone by STGT, $F(1, 544) = 0.00$, $p>.10$, Tone by Intake, $F(1, 544) = 0.78$, $p>.10$ or STGT by Intake, $F(1, 544) = 0.01$, $p>.10$ Significant three-way interactions were found for Response by STGT by Intake, $F(1, 544) = 12.28$, $p=.001$ but not for

Tone by STGT by Intake, $F(1, 544) = 1.03$, $p>.10$ Tone by Response by STGT, $F(1, 544) = 0.02$, $p>.10$ or Tone by Response by Intake, $F(1, 544) = 0.61$, $p>.10$. The four-way interaction was not significant, i.e., Tone by Response by STGT by Intake, $F(1, 544) = 0.00$, $p>.10$. Therefore, we found no evidence of tone-evoked activity differences in GT or Non-GT (baseline vs post).

Follow up simple effects F-tests were run to analyze the differences between Hit vs. Miss (Response) for all possible combinations of STGT and Intake. Baseline and Posttone FR was collapsed to 400ms to capture the entire pre and post-tone window (because the above analyses had revealed no difference between the two). The distribution of ‘silent’ (0 FR average for a session) vs ‘active’ (Non-0 average session firing rate) revealed significantly more ‘active’ neurons for Hit trials vs Miss trials in the GT-High Intake group, $F(1, 544) = 54.54$, $p. < .0001$. Differences in Response for all other subgroups were not significant, GT-Low Intake, $F(1, 544) = 1.33$, $p. > .10$, Non-GT-High intake, $F(1, 544) = 1.17$, $p. > .10$ and Non-GT Low intake $F(1, 544) = .06$, $p. > .10$. Thus, there was a significantly different ‘logistic’ pattern of activity in High intake GT shell neurons. During Hits, neurons were already ‘active’ and remained active, but on Misses the neuron stayed quiet and did not change in response to the tone (figure 8b).

Although Tone was not found to be significant in the logistic Baseline versus Posttone analysis, there was apriori interest in addressing the overall distribution of non-zero firing rates. Further analysis of Nac shell ‘active’ neural distributions (i.e., the non-zero neuron-sessions) revealed group findings nearly identical to the above logistic analysis. A gamma mixed ANOVA model was used to analyze

the continuous distribution of the non-0 average FR values for Shell neurons, i.e., active average FRs pre vs post tone. The gamma model showed a significant main effect of Response, $F(1, 301) = 10.28$, $p=.002$, but not of Tone, $F(1,301)=0.16$, $p>.10$ or STGT, $F(1, 301) = 2.37$, $p>.05$. A significant two-way interaction was found only for Response by Intake, $F(1, 301) = 8.05$, $p=.005$. All other two way interactions were nonsignificant: Tone by Response, $F(1, 301) = 3.69$, $p>.05$, Tone by STGT, $F(1, 301) = 0.75$, $p>.10$, Response by STGT, $F(1, 301) = 0.28$, $p>.10$ Tone by Intake, $F(1, 301) = 1.12$, $p>.10$, and STGT by Intake, $F(1, 301) = 0.00$, $p>.10$. Additionally, a significant three-way interaction was found only for Response STGT by Intake, $F(1, 301) = 4.50$, $p=.035$. All other three-way interactions were non-significant; Tone by STGT by Intake, $F(1, 301) = 0.11$, $p>.10$, Tone by Response by STGT, $F(1, 301) = 1.10$, $p>.10$, Tone by Response by Intake, $F(1, 301) = 0.00$, $p>.10$. The four-way interaction was not significant Tone by Response by STGT by Intake, $F(1, 301) = 0.25$, $p>.10$. These results reaffirm the absence of tone-evoked changes in FR during Maintenance of cocaine SA.

Because of the significant three-way interaction of Response by STGT by Intake, follow up simple effects F-tests were run to analyze the 400ms FR differences (collapsing Baseline and Posttone) between Hit vs. Miss for all possible combinations of STGT and Intake. The difference between Hit vs. Miss was significant for the GT-High Intake group, $F(1, 301) = 30.36$, $p. < .0001$. There were no differences between Hit vs. Miss for all other subgroups, GT-Low Intake, $F(1, 301) = 1.00$, $p. > .10$, Non-GT-High, $F(1, 301) = 2.10$, $p. > .10$ and Non-GT-

Low $F(1, 301) = .58$, $p. > .10$. An important result in this case involves the similarity of the binary distribution (above), in that FR distributions were significantly different on Hits versus Misses for High intake GT shell neurons that were ‘active’. This segregation of FRs was not found in other subgroups, indicating that underlying shell activity was different prior to behavioral responses (drug seeking vs abstaining), but only in High intake GT.

Thus, shell neurons showed no evidence of tone-evoked firing in any group, but rather, evidence of underlying logistic differences on Hits versus Misses in High intake GT. Neurons that were ‘silent’ on Misses were ‘active’ on trials that resulted in a Hit. Assessment of already active neurons demonstrated that these different FR distributions on Hits vs misses were observed only for GT high intake.

5 Discussion

5.0*:* Historically, Goal-Trackers have been largely ignored in drug addiction studies, overshadowed by a common focus on their Sign-Tracking counterparts. However, GT have been shown to ingest drugs at similar rates across days (Saunders & Robinson, 2010), and self-administer the same amount of drug at low doses (Bardo et al, 2006). The present study reaffirmed that increased lever pressing (sign-tracking) during the STGT pretest did not predict any increase in drug consumption (figure 4ab), We also found that both GT and Non-GT animals demonstrated selective tone-discrimination in the SA task during pre-drug trials (figure 6a). Further, we identified similar escalation of drug intake in GT and Non-GT animals (Figure 5) and similar rates of high intake sessions (table 2). Escalation of drug intake is a key measure of addiction in animal models (Ahmed & Koob, 1998). Together, our findings indicate that GT and Non-GT as a population are equally likely to use drugs, discriminate drug-cues, and have similar representation of the high risk behavior (i.e., high intake) for drug addiction despite literature bias towards ST.

5.1: Chronic drug self-administration is also known to involve negative reinforcement. During the Maintenance phase of cocaine self-administration, animals will compulsively seek drugs at ‘sub-satiety’ levels, but cease drug responding when DL is clamped at or above satiety level (Barker et al., 2014). In fact, studies suggest that animals ‘titrate’ around a preferred drug-level during binges by taking drugs when DL is low and ignoring drugs/drug-cues when DL is high (Barker, et al. 2014). This method of self-administration appears to both

escape or prevent withdrawal, and maintain a ‘preferred’ DL within the session. This normally results in minimal fluctuations in internal drug level from the ‘desired’ mean in part because too high or too low drug level has been shown to be aversive (Blanchard & Blanchard, 1999). Our 6 hour SA paradigm utilized 1-6 minute variable ‘drug timeout’ periods (ITI), which created variable periods of drug unavailability, designed so that 50% of trials occurred when DL was above, and the other 50% when DL was below, the animal’s ‘desired’ cocaine level. One goal of the present design was to enable animals to experience both satiety and sub-satiety DLs, but the paradigm may have exposed an underlying sensitivity to ‘unpredictable’ reward availability in GT animals. Random (variable)-intervals are more anxiety inducing than fixed intervals in operant conditioning and elicit faster response rates than fixed interval conditioning (McLeod, 2015). Cocaine at high levels has been shown to elevate anxiety-states in animals, and GT high intake had the highest DL on Hits relative to all other groups’ Hits or Misses (figure7a) (Blanchard & Blanchard, 1999). Although we found STGT is not a predictor of initial drug-use, it is known that not all humans who use drugs will go on to develop drug addiction. Instead, because preference for high drug intake is a classic DSM characteristic of drug addiction (Wolffgramm, 1995) it is more clinically relevant to identify individual predisposition to develop *severe* drug addiction. Although High intake GT and Non-GT exhibited similar prevalence of high intake sessions (table 2), we identified potentially dangerous patterns of drug responding among high intake GT that did not appear in high intake Non-GT in response to the randomized drug unavailability of the experiment. Given that

addiction may not be a ‘one size fits all’ diagnosis, we speculate that GT and Non-GT animals are prone to develop two different types of drug addiction based on their inherent phenotype (i.e., goal, vs cue oriented):

5.2: Differences in High Intake GT vs Non-GT

GT: Unexpectedly, high intake GT animals responded contrary to titration logic. They Hit when DL was already high, which raised their drug level to higher, more dangerous levels, while their Miss DL was much lower. Despite selective tone-discrimination during drug-free trials earlier that same day (figure 6a), high intake GT abandoned tone-discrimination during the ‘drug-onboard’ maintenance phase of cocaine SA. During the ‘drug-onboard’ maintenance trials, GT high intake correlated with high levels of indiscriminate drug seeking during the ‘tone-off’ period when drug was unavailable (figure 6cd). These results suggest that higher drug intake in GT animals was driven by accelerated rates of overall drug seeking, irrespective of drug availability signaled by the tone, possibly associated with hypersensitivity to internal drug cravings. GT high intake were also unable to ‘control’ their internal drug level fluctuations during the session (figure 7a) and this resulted in significantly higher DL on Hits (relative to Misses) (figure 7b). During the cocaine-binge, GT took large quantities of drug and were willing to tolerate high DL to avoid situations where they could be craving drugs for an uncertain amount of time (for example, during a run of several long ITIs in a row). This avoidance technique is consistent with the ‘negative reinforcement’ theory of addiction, and suggests a dangerous predisposition to accidentally overdose for high intake GT. Likewise, high intake

GT Shell and Core Neurons did not show tone-responsiveness on Hits or Misses, i.e., did not *change* in response to the tone (figure 8ab). Instead, neurons overwhelmingly stayed silent on Misses (i.e., Baseline and Posttone), but the same neurons were already active (baseline) and remained active (Posttone) on Hit trials during the same session (Figure 9ab). Thus, Nac activity differences were found to be correlated with behavioral outcomes, i.e., drug-seeking (FR active) vs abstaining (FR silent), in animals that represented risk for severe drug addiction and overdose (high intake GT).

Non-GT responded to the variable ITI as expected and titrated their drug level effectively during the task: Hit when DL was low, Miss when DL was high, and thus DL did not dramatically fluctuate within session. Non-GT animals did not exhibit higher rates of 'uncued' drug responding during maintenance trials when drug intake was high (figure 6c) nor when Hit% increased (figure 6d). These results suggest that Non-GT maintained selective tone-discrimination throughout the SA task. Non-GT animals (high and low intake) were able to titrate their DL during the task in response to drug availability signaled by the tone: taking Hits when DL was low and Miss when DL was high (figure 7b). As a result, their drug levels did not dramatically fluctuate within session between Hits and Misses (figure 7a). This suggests that Non-GT animals' increased drug intake was driven by additional selective tone-responding when the animal craved drugs, and not general drug seeking. Non-GT may be predisposed to incentivize drug-cues predictive of drug availability, in that the tone signaled drug much like the lever signaled sucrose reward during

the STGT pretest. This selective tone-responding may have been reflected in their Nac neurons' FR. On Hits, when the animal chose to take drug, Core neurons' FR changed in response to the tone, but the same neurons did not change on Misses (figure 8a). Shell neurons did not respond to the tone at all during Maintenance. Collectively, this suggests that Non-GT animals develop a drug strategy consistent with being reactive to external cues, and thus respond for drugs when they are available, to 'titrate' effectively within the session.

5.3: Neural correlates to behavior, Hits vs Misses: The purpose of this experiment was to identify tone-evoked FR patterns of Nucleus Accumbens neurons when the animal sought drug in response to the tone (Hit) or ignored drugs (Miss) in well-trained animals prescreened for GT and Non-GT. During Maintenance, Shell neurons showed no evidence of 'tone-evoked' changes in, FR. Core neurons showed some tone-evoked changes in High intake Non-GT: Posttone FR was significantly different from Baseline FR on Hits, but not on Misses (figure 8ab). This is consistent with the possibility that Nac neurons contribute to neural networks that react to exteroceptive cues, such as the lever during the pretest. High intake GT Nac Core and Shell neurons did not show 'tone evoked' changes on Hits or Misses (figure 8ab), consistent with lower empirical measures of sign tracking in GT (low lever contacts during pretest). Instead an interesting pattern emerged in GT in which the same neuron that was 'silent' Baseline and Posttone on Misses became 'active' Baseline and Posttone on Hits during the same session (figure 9ab). The general silence vs activity was

predictive of the behavioral outcome (i.e., drug-seeking vs abstaining from cocaine use).

Together, it appears the Nac shows similar involvement in both GT and Non-GT with regard to avoiding opportunities to take drug (Miss). When the tone was presented and the animal did not take/want the drug (Miss), the neuron did not change in firing, and in GT the neurons remained overwhelmingly 'silent'. Important differences in Nac FR were found on Hits. Non-GT Core neurons showed cue-evoked activity on trials that led to a Hit, but GT Core and Shell neurons remained 'active' selectively during Hits, suggesting that GT may have been predisposed to take drug at the next opportunity, potentially because of elevated FR of Nac neurons. These patterns of activity suggest that Nac neurons are involved in motivation to seek drug. On Hits, Non-GT change FR in response to the S^D while GT neurons are already 'active'. On Misses, both GT and Non-GT neurons do not change, and in fact, GT neurons remain completely silent. Thus, we found evidence of a large sample of Nac activity/inactivity patterns that directly correlated with drug taking (Hits, active neurons) and avoiding (Miss, silent neurons), possibly as a function of drug craving, particularly in animals that represent a novel form of drug addiction, high intake GT.

5.4 Summary and Conclusion: Drug addiction does not discriminate. It impacts people of all walks of life (Grant *et al.,* 2006), and it is likely that individual differences enable heterogeneous neurobehavioral formations of drug abuse phenotypes. It is also true that not all drug users go on to develop addiction, but preference for high drug intake is known as a warning sign for future addiction

(Wolffgramm & Heyne, 1995). STGT phenotypes generalize to humans (Garofalo & diPellegrino, 2015) and although we found STGT does not predict overall drug consumption (figure 4ab), we identified high-risk behavioral and Nac firing patterns unique to High intake GT animals which were not present in high intake Non-GT animals (or any low intake group):

High intake **Non-GT** self-administer drugs in response to cue-signaled drug availability, evidenced by low uncued drug seeking (figure 6cd) and minimal fluctuations in DL within session (figure 7ab). Non-GT Core neurons also showed tone-evoked changes on Hits, but not on Misses (figure 8ab). Therefore, Non-GT may incentivize the tone-cue similarly to the lever-CS which signaled sucrose reward during the STGT pretest. For example, ST animals may incentivize inconsequential local stimuli (eg, the CS-lever in the STGT pretest).

High intake **GT** take large quantities of drugs, possibly to circumvent periods of unavailable drug. For GT, avoiding periods of intensive drug craving may be the goal they are actually tracking, responding irrespective of the external tone-cue, which was more effectively tracked by Non-GT. GT increased DL on Hits to potentially dangerous levels (figure 7b), possibly to avoid future periods when they may be below a ‘desired’ DL and without access to drug rewards, a condition which has been demonstrated to be aversive (Barker et al, 2014). Together with their high rate of uncued drug seeking (figure 6cd) these findings suggest they were already ‘prepared’ for the next infusion and were compulsively drug seeking. Reflecting this craving, GT neurons were already ‘active’ prior to tone onset and stayed active (not changing in response to the tone) on Hits,

contrasted with their silence on Misses (figure 8ab). This suggests a type of drug addiction formation which could be correlated with a lethal combination of high drug tolerance and accidental overdose (two additional aspects of DSM-5).

We speculate that GT animals are predisposed to develop a unique (and dangerous) type of drug addiction relative to Non-GT animals of similar high intake. GT animals fixate their behavior on all necessary cues to achieve their goal, such as the food port in the STGT pretest. During drug self-administration, GT could fixate on internal drug cues (such as falling drug level), urges, or cravings and seek drugs to avoid periods of drug unavailability. As a result, GT are able to tolerate (or are less sensitive to) high drug levels to circumvent those situations. Therefore, high intake GT may represent a severe goal-oriented model of ‘high-risk’ drug addict because they are particularly focused on avoiding the negative ‘potentially unavailable’ drug situations such that they risk overuse, and overdose, to prevent that situation from happening.

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7 Figures

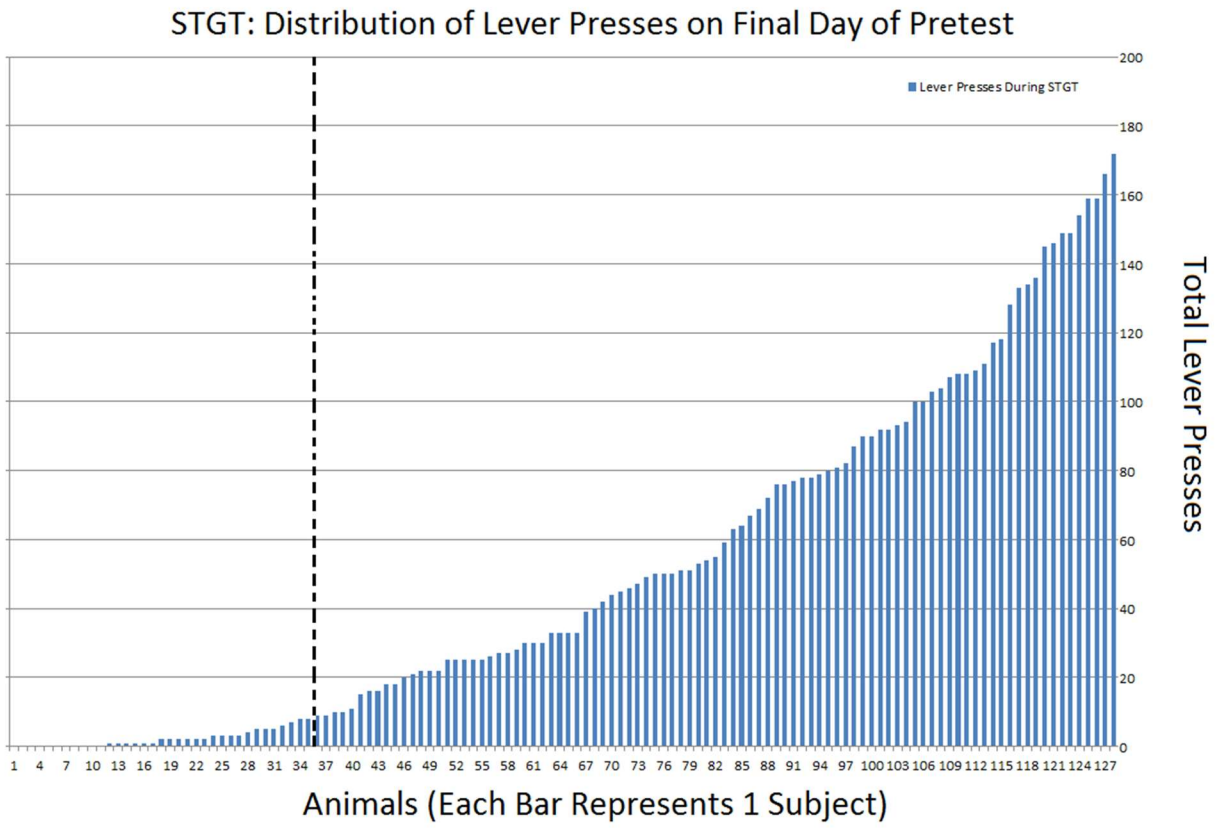

Figure 1: STGT distribution based on final pretest lever-press data, N=147. Animals selected at <35 LP, representing the lowest 20% of lever pressers, were defined as possessing strong GT traits. Animals exhibiting >35 LP were defined as Non-GT (weak goal-tracking traits).

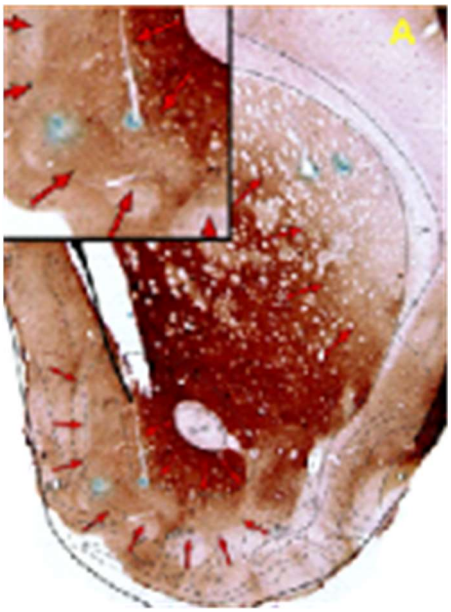

Figure 2: Histological identification of Nac core and shell. Nac core (calbindin heavy) and Nac shell (calbindin-light) neurons were recorded and analyzed with respect to different behavioral responses during cocaine self-administration. Wires from regions outside of this area were recorded, but discarded for all analyses purposes. Arrows indicate the border of Nac shell

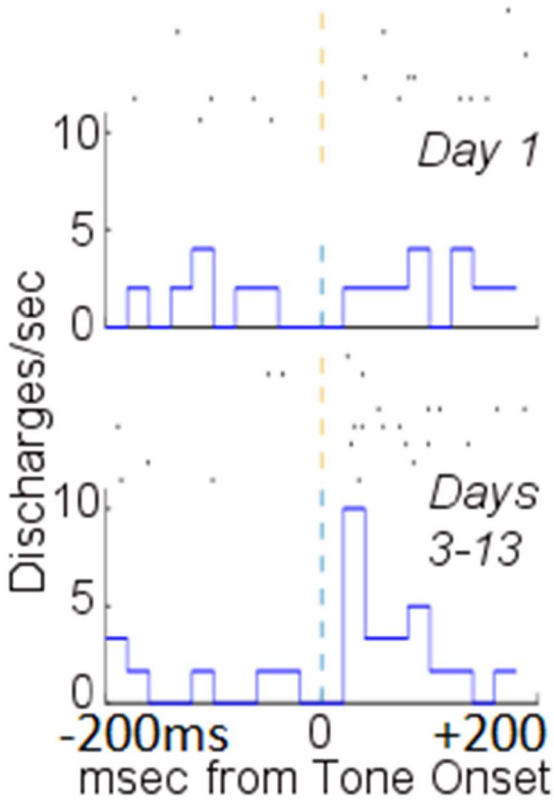

Figure 3. Tone evoked FR of one neuron during 'Pre' drug trials. Neuron shows weak FR on day 1 and stronger FR as the sessions advance. Tone is defined as -200ms=baseline FR, +200ms=post FR. Firing rate data are averaged across different behavioral responses to produce a 'neuron-session.' Interestingly, minimal tone-evoked activity was observed, except for High Non-GT (and low-intake GT) Core neurons. No Shell neurons demonstrated any activity differences Baseline versus Posttone during maintenance trials, which were the focus of the present study.

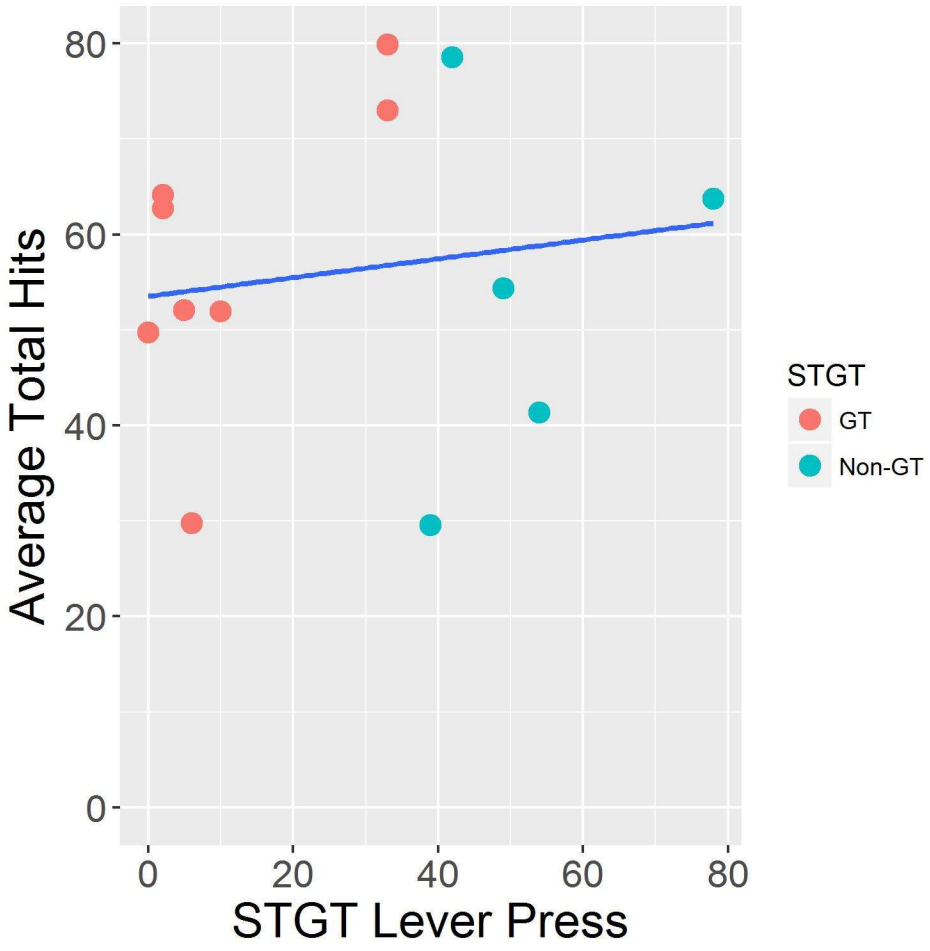

Figure 4a: Average Hits (via nosepoke) during cocaine SA did not increase as a function of lever pressing on the final day of the STGT pretest. The slope for Hits X STGT Lever Press (b = 0.098) was not significantly different from 0, t(1) = 0.50, p > .10 revealing that Average cocaine intake is stable as a function of the "sign-tracking" phenotype.

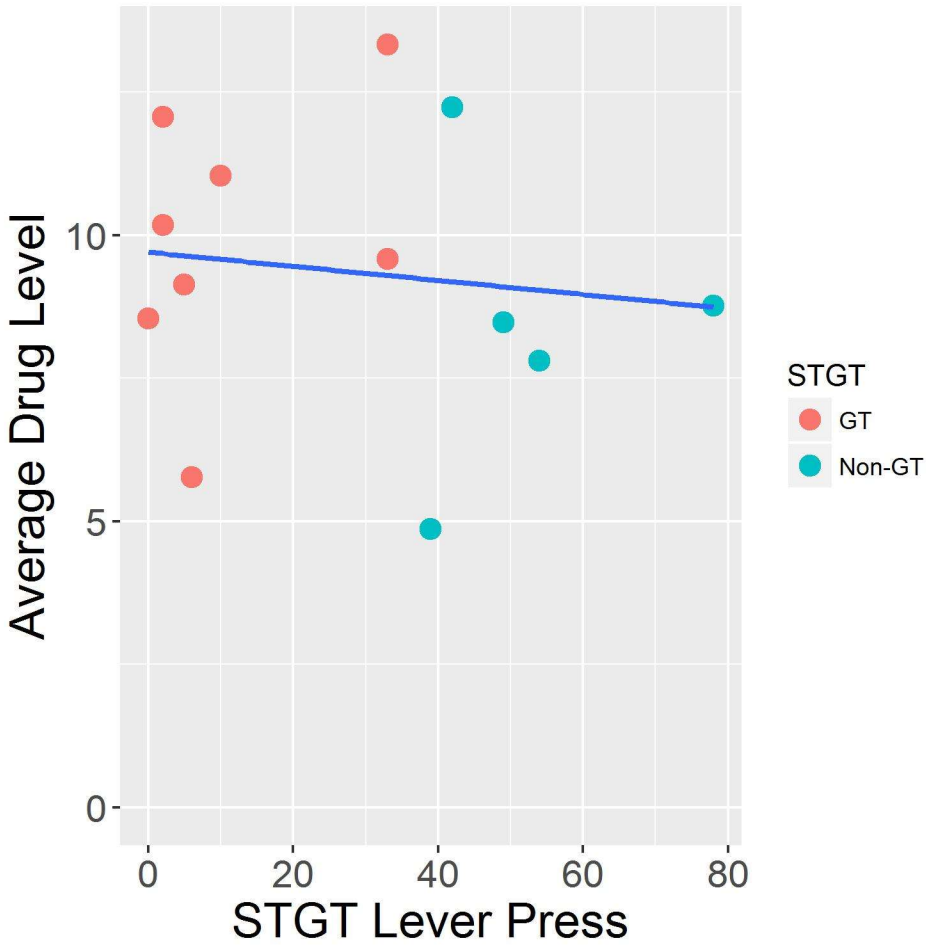

Figure 4b: Average drug level as a function of Pretest Leverpressing. Each dot represents the average drug level of individual animals during late cocaine sessions. The slope for Hits X STGT Lever Press (b = -0.01) was not significantly different from 0, $t(1) = -0.42$, $p > .10$. There is no increase in drug level as a function of pretest "sign-tracking."

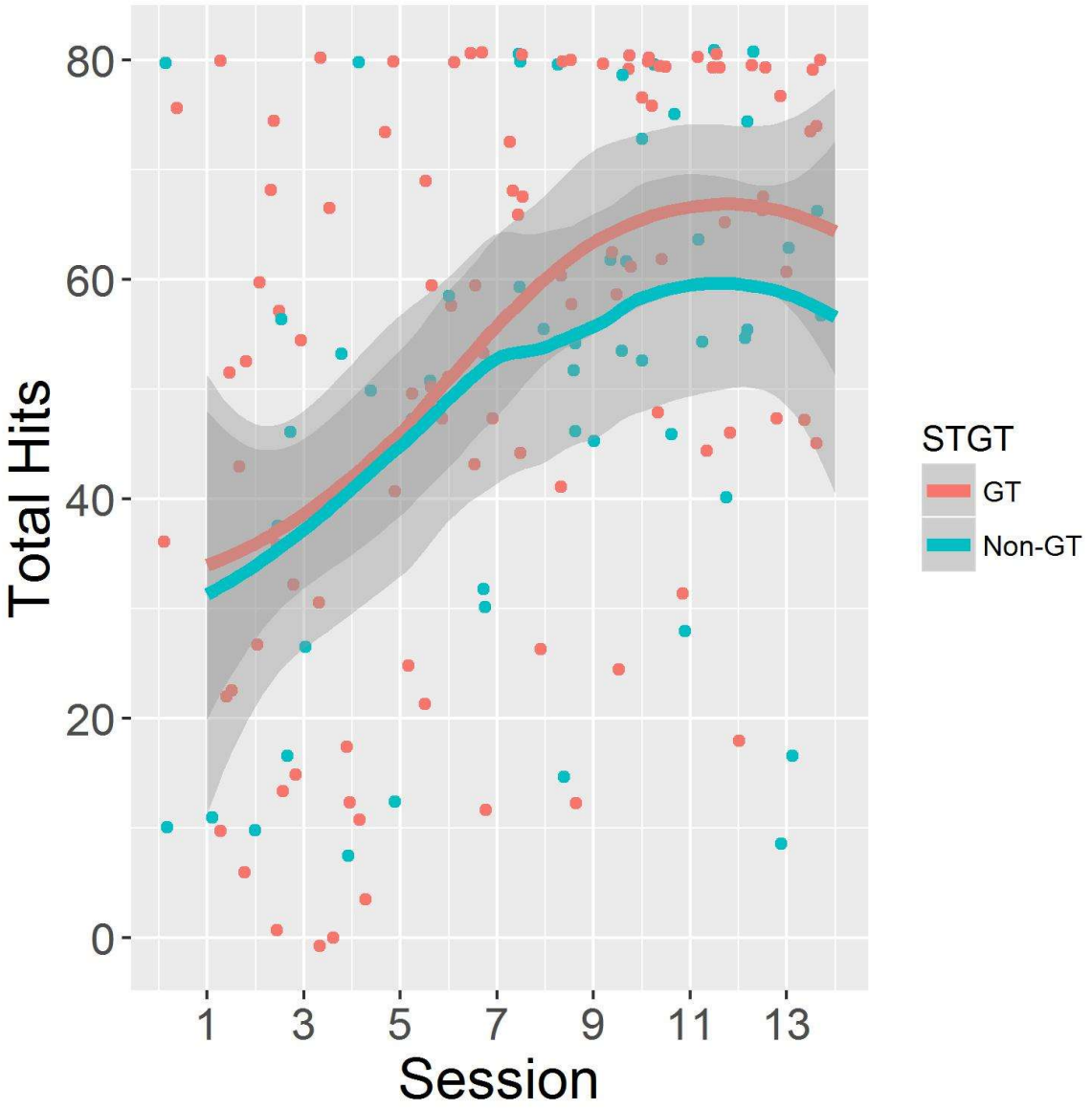

Figure 5: Escalation of Cocaine Intake. Each dot represents one subject's total Hits for each session across 14 days of cocaine SA. Both GT and Non-GT demonstrate escalation of drug intake over sessions; Session, $F(1, 113) = 10.56$, $p = .0015$, which suggests cocaine acquisition, but not a significant main effect of STGT, $F(1, 113) = 0.53$, $p. > .10$, nor a significant interaction of Session by STGT, $F(1, 113) = 1.52$, $p > .10$. Therefore, both populations acquired cocaine self-administration and escalated intake at similar rates.

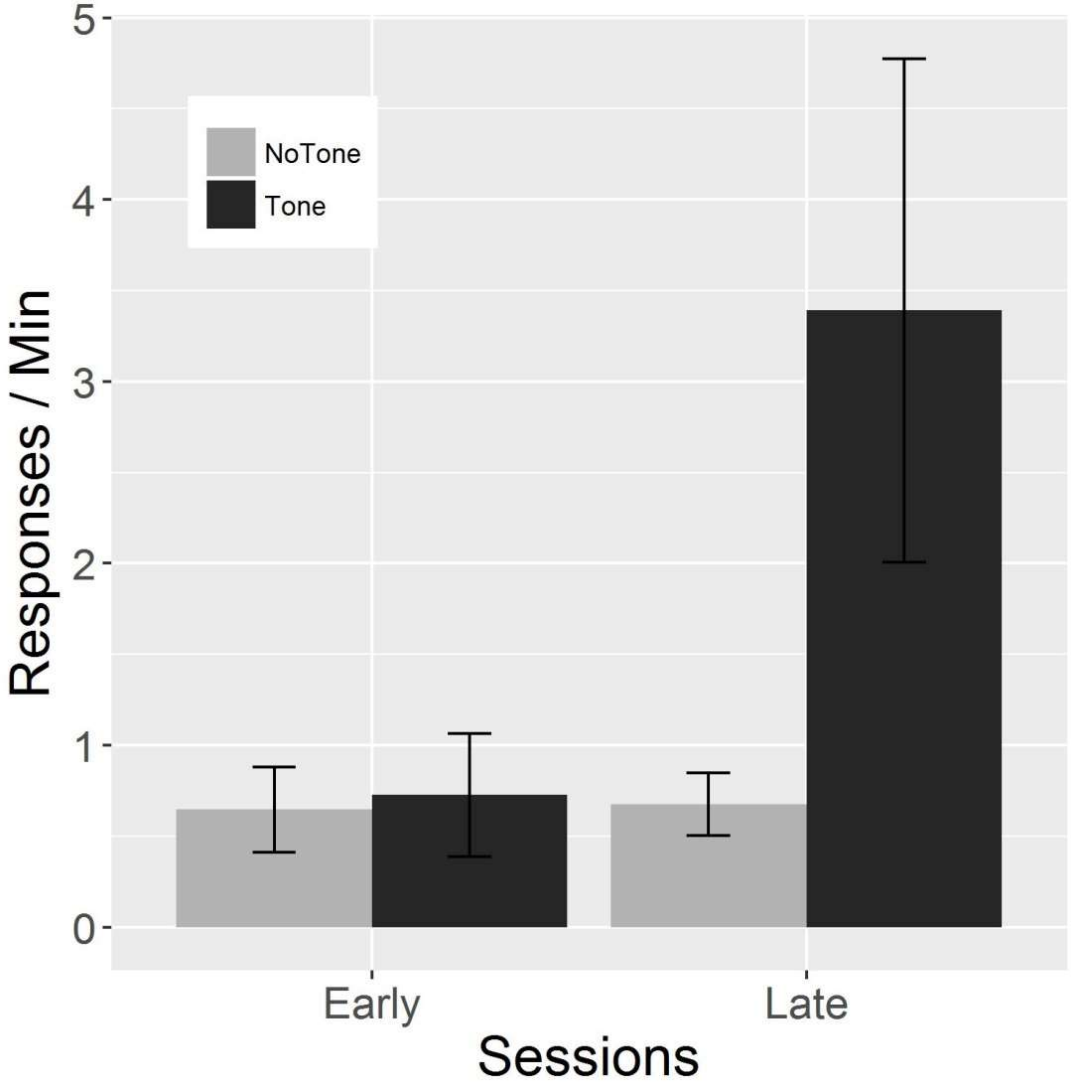

Figure 6a: Response-Rate of Cued (tone-on, drug available) and Uncued (tone-off, drug unavailable) drug seeking during all drug-free trials leading to the first cocaine infusion of the day, prior to resumption of drug taking. Cued responding increased equivalently in GT and Non-GT across sessions while Uncued responding was stable in GT and Non-GT and both did not increase. Significant results were found for Session Epoch (early vs. late) by Response-Rate (Cued v Uncued), $F(1, 327) = 6.41$, $p = .012$, but not for Session Epoch by STGT, $F(1, 327) = .66$ $p > .10$, nor for Response-Rate by STGT $F(1, 327) = .00$ $p > .10$. Therefore, because Cued (but not Uncued) responding increased at similar rates in both GT and Non-GT animals it is clear that both groups exhibited similar acquisition of selective tone-discrimination during drug-free trials. Therefore, both GT and Non-GT are equally likely to discriminate operant cues associated with drug reward prior to drug relapse.

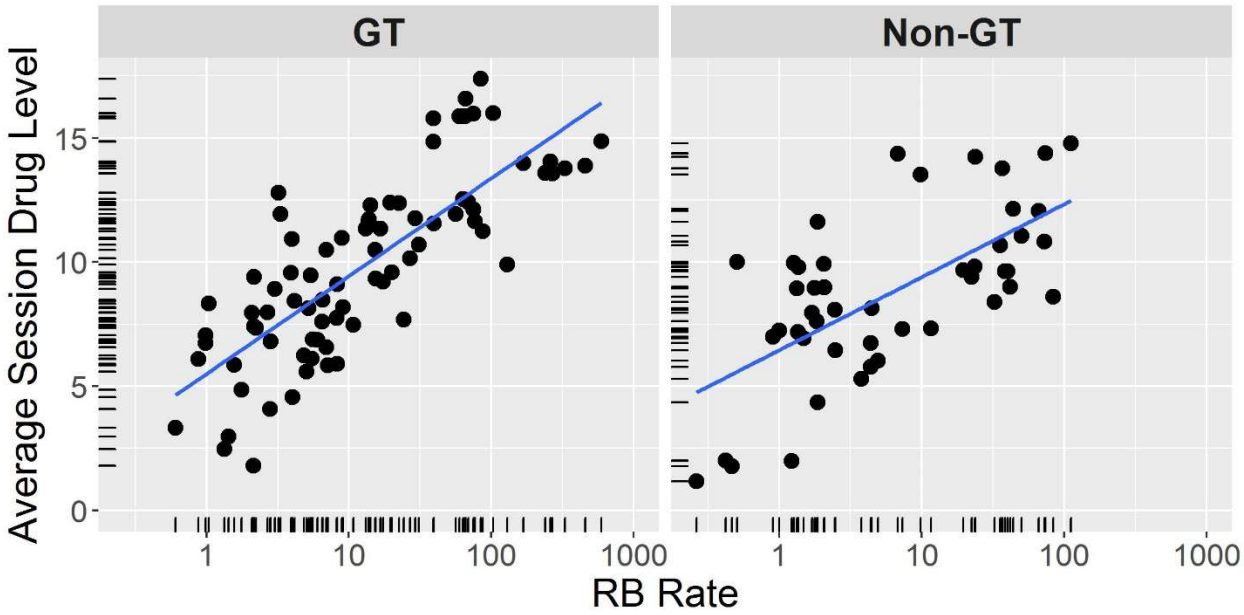

Figure 6b: Rates of Uncued drug seeking during Maintenance (drug-onboard). Average drug level increased as a function of higher rates of uncued drug seeking (RB Rate) during cocaine self-administration. Uncued drug seeking was correlated with an increase in drug level in both GT *r*=.76 and Non-GT *r*=.65 when addressing both populations (and collapsing across intake categories). However, in order to address if this uncued drug seeking was different at the highest and lowest intake (high Intake=>55 Hits), two segregated analyses were performed below (figure 6c & d).

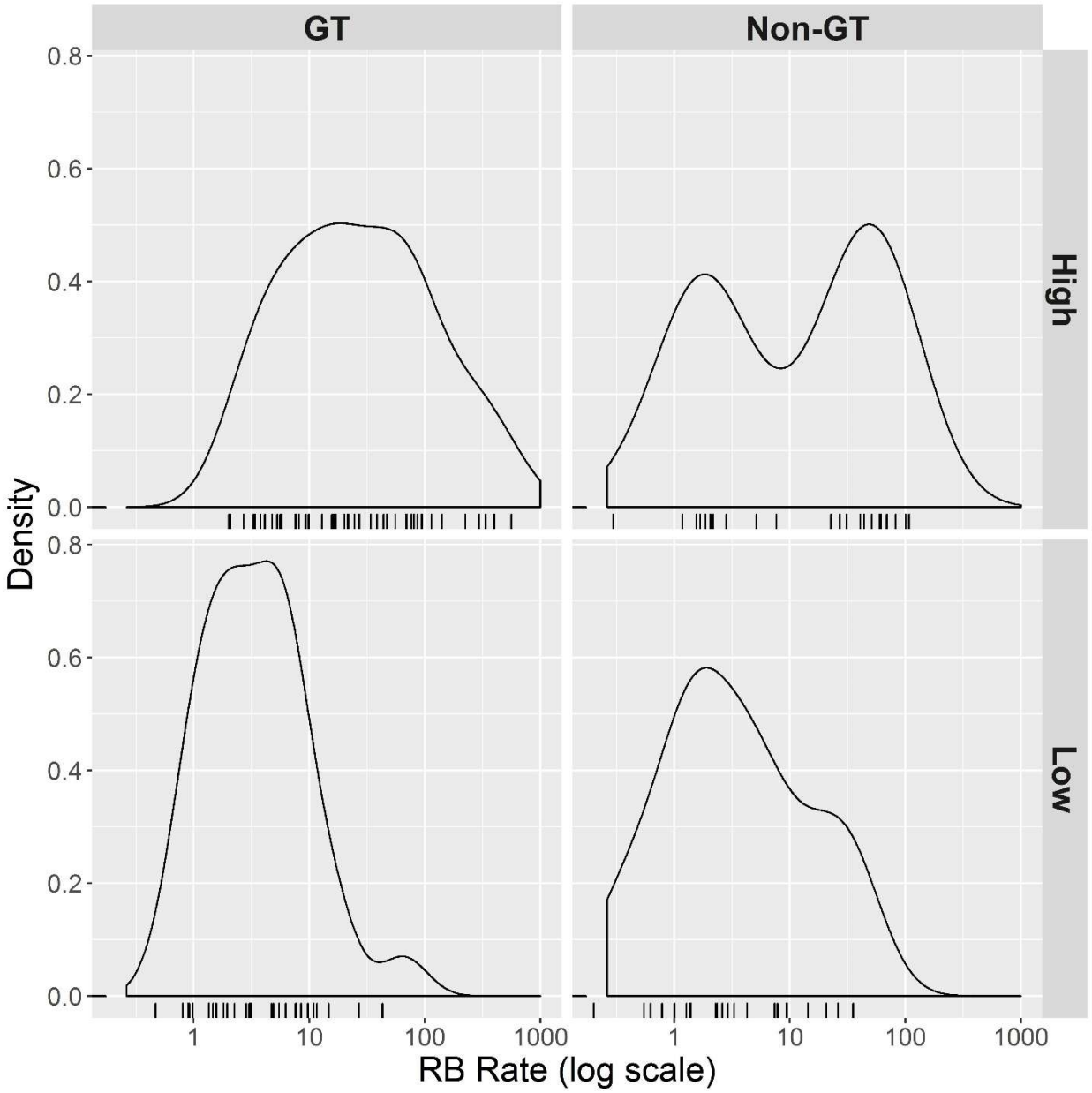

Figure 6c: Density plots show rates of Uncued Maintenance drug seeking differs among STGT and Intake category: For GT animals, uncued drug seeking (Tone Off, Drug Unavailable) was significantly different between high and low intake $F(1,75) = 14.21$, $p = .0003$, but not for Non-GT high vs low $F(1,44) = 1.32$, $p > .10$. These findings were also true for the subset of animals that exhibited sessions of both high and low drug intake (i.e., after removing animals that contributed to only one or the other). GT high vs low intake differed significantly $F(1,45) = 1.32$, $p = .0043$ whereas Non-GT did not $F(1,27) = 1.01$, $p > .10$. Therefore, it appeared that high drug intake for GT was driven by compulsive drug seeking irrespective of the drug availability signaled by the tone, while Non-GT high intake was driven by additional selective responses during the tone.

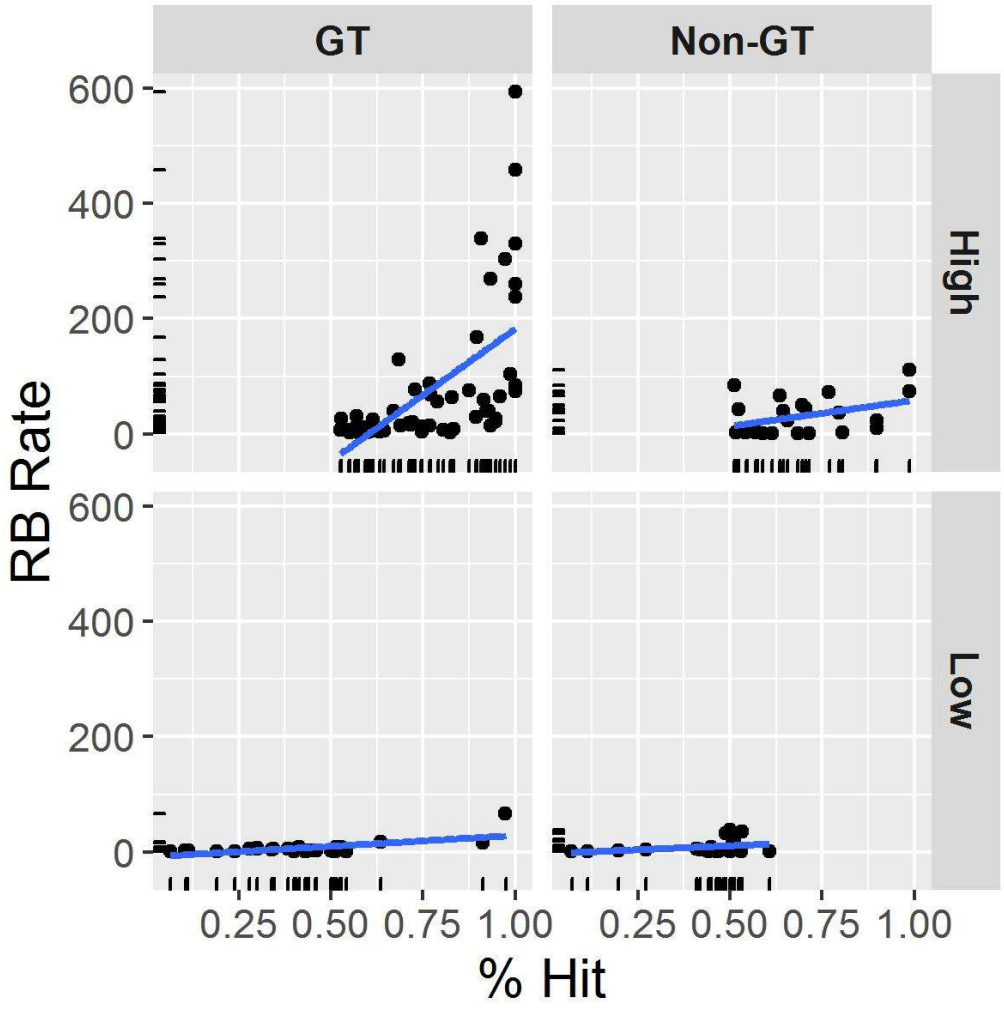

Figure 6d: Uncued drug seeking regressed on %Hit exhibited a different slope in High intake GT, Analysis of uncued drug seeking slope differences among the four categories revealed that High intake GT showed significantly higher slopes relative to every other group. First, all slopes were significantly different from zero: GT-High, B = 4.67, t(112) = 12.22, p < .0001, GT-Low subgroup, B = 3.41, t(112) = 5.98, p. < .0001, Non-GT-High subgroup, B = 2.65, t(112) = 3.06, p = .0028, for the Non-GT-Low subgroup, B = 4.33, t(112) = 3.71, p. =.0003. However, it was evident that these slopes were not identical at higher intake for GT, and post-hoc that uncued seeking slopes were at the different extremes of intake. Post-hoc comparisons of GT vs. Non-GT subgroups for High Intake showed a significant difference, t(112) = 2.13, p. = .035, but not between Low Intake GT vs. Non-GT , t(112) = -0.71, p > .10. These findings suggest higher GT drug intake may be driven by compulsive drug seeking, and possibly strong craving for drug. Non-GT, conversely, achieved high intake via selective tone-discrimination responding.

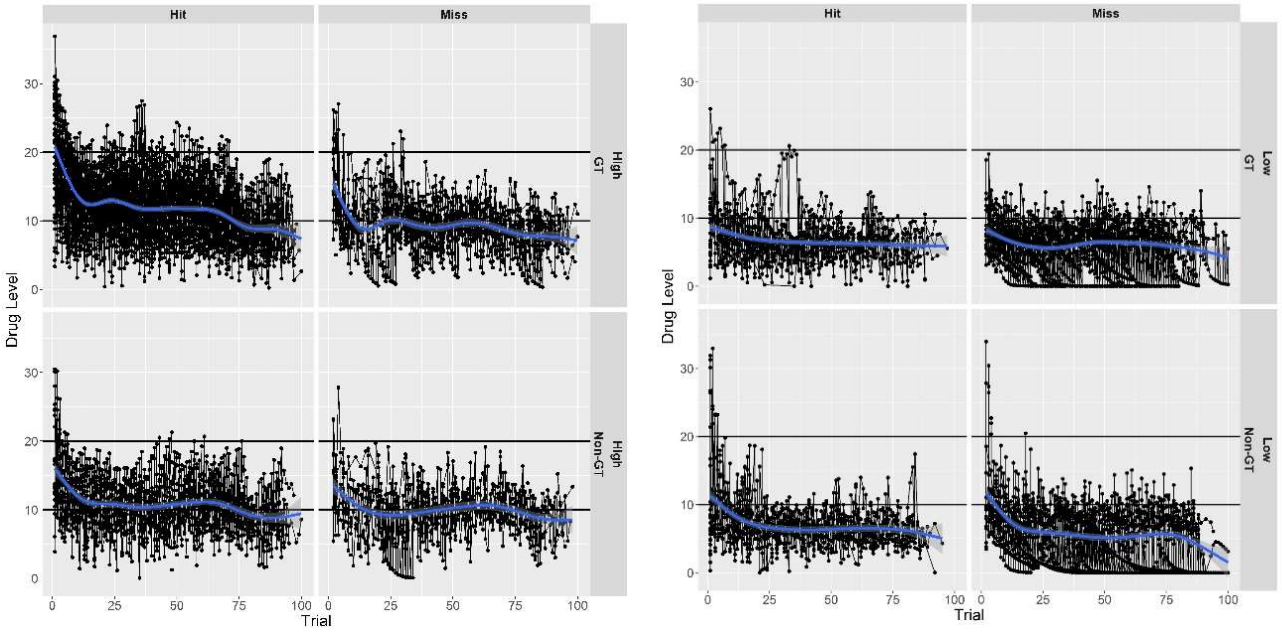

Figure 7a: Drug level differences on Hit vs Miss trials:

GT and Non-GT animals on high intake days. An assessment of within session drug level variability between hits and misses revealed a significant three way interaction for Response (Hit/Miss) by STGT (GT/Non-GT) by Intake (High/Low) $F(1, 10586) = 4.16$, $p = .041$. Follow-up simple effects tests revealed Hit and Miss DL was significantly different for only one subcategory; GT High intake, $F(1, 10586) = 15.85$, $p. < .0001$, indicating high intake GT animals had significantly different Hit drug level compared to their Miss drug level during the same session. No other subgroup was statistically different miss/hit drug level; GT Low Intake, $F(1, 10586) = 0.15$, $p. > .10$, Non-GT High intake, $F(1, 10586) = 0.95$, $p > .10$ Non-GT Low intake, $F(1, 10586) = 0.55$, $p > .10$. Thus the majority of animals were able to control (titrate) their drug intake. In contrast, GT high intake are unable to control their drug intake, and drug level fluctuated dramatically on different behavioral opportunities (Hits v Misses).

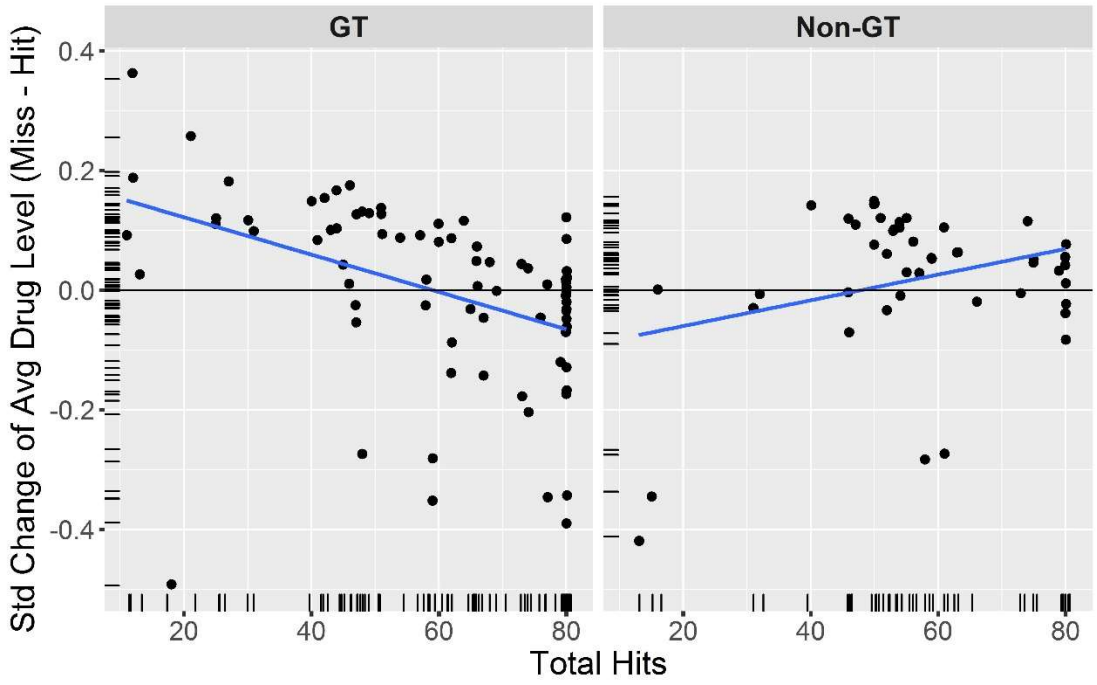

Figure 7b: Lack of titration in GT animals. Due to our interest in self-administration patterns amongst the highest GT and Non-GT, and between-group differences in Hit vs Miss DL, we specified three levels of intake on the continuum of Total Hits. This captured the full range of the continuous IV at the lowest, median, and highest drug intake thresholds (lowest = 10, medium = 45, highest = 80). Standardized change scores of drug level between Hit/Miss (MissDL-HitDL)/(MissDL+HitDL+.1) were calculated to assess drug level differences on Hits and Misses in GT and Non-GT. Literature indicates animals take drugs to seek a "preferred" drug level within a given session. Animals typically self-administer drug (Hit) when drug level is below this 'desired' level and ignore cocaine opportunities when the drug level is high (Miss) (Root 2009; Root et al, 2011). Based on titration literature, a positive SC value was expected. A negative SC value was unexpected and would be contrary to literature, indicating a low Miss DL and high Hit DL in the same session. As expected, at the lowest intake, GT had higher Drug level SC for Misses, $t(109) = 2.23$, $p = 0.028$ and analysis of moderate intake showed no difference in GT and Non-GT Drug Level SC, $t(109) = .55$, $p > .10$. In contrast, analysis of the highest intake revealed GT had a significantly lower (and negative) DL change, while Non-GT had a positive change $t(109) = -2.18$, $p = 0.0318$. This result is unexpected to titration logic, and indicates that as a function of increasing Total hits (intake) GT had a lower Miss/Hit DL, while Non-GT had an inverse pattern consistent with titration (high Hit/Miss DL).

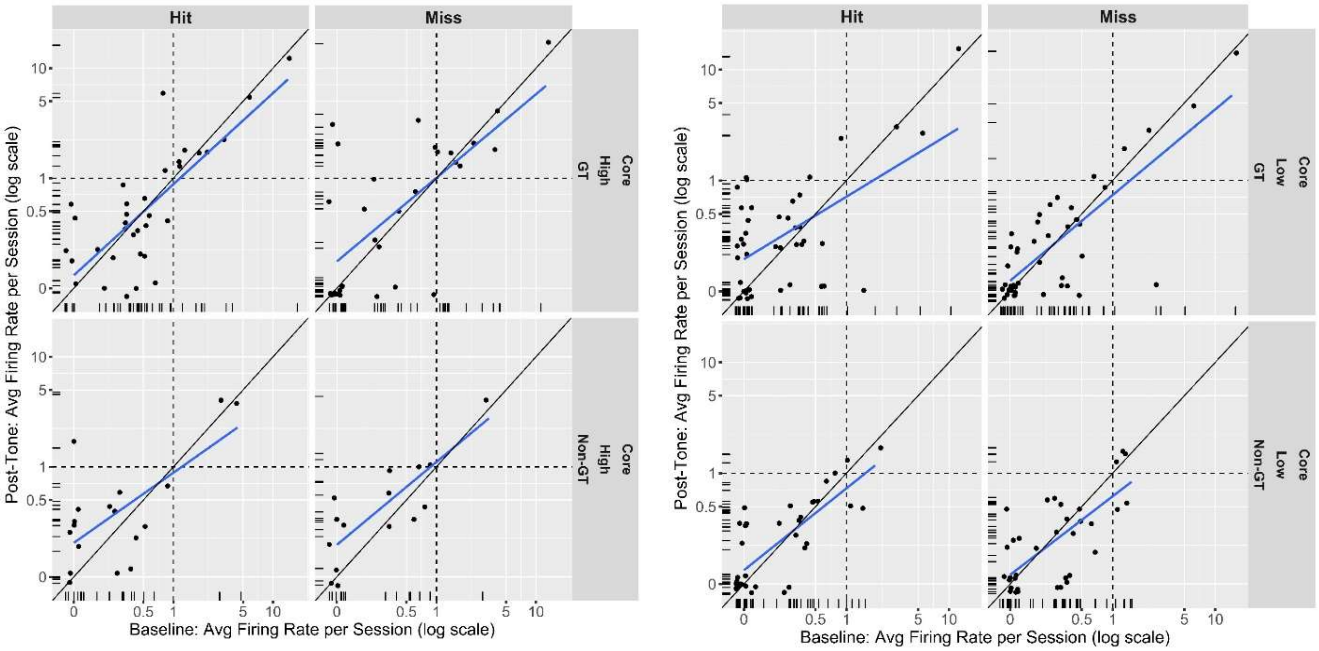

Figure 8a: Assessment of tone-evoked activity differences in Core FR. What is the effect of baseline vs. posttone (TONE), are the FRs different across Hits vs Misses (RESPONSE) and does this differ across GT vs Non-GT (STGT)? For Core neurons, stability between Baseline and Post-Tone FR was maintained for all conditions and significant positive correlations for all neuron-sessions (table 1). However, an analysis of the distribution of zero and nonzero in Baseline vs Post revealed evidence of Tone-evoked activity for the Non-GT-High Intake group, $F(1, 447) = 8.23$, $p. = .004$ and for the GT low Intake group $F(1, 447) = 4.39$, $p. = .037$. The differences between Tone for all other subgroups were not significant, GT-High, $F(1, 447) = 0.00$, $p. > .10$ and Non-GT Low $F(1, 447) = 0.00$, $p. > .10$. Thus, core neurons demonstrated tone-evoked activity in Non-GT high intake animals and in GT low intake animals, but this did not appear to be the case for GT high intake nor Non-GT low intake.

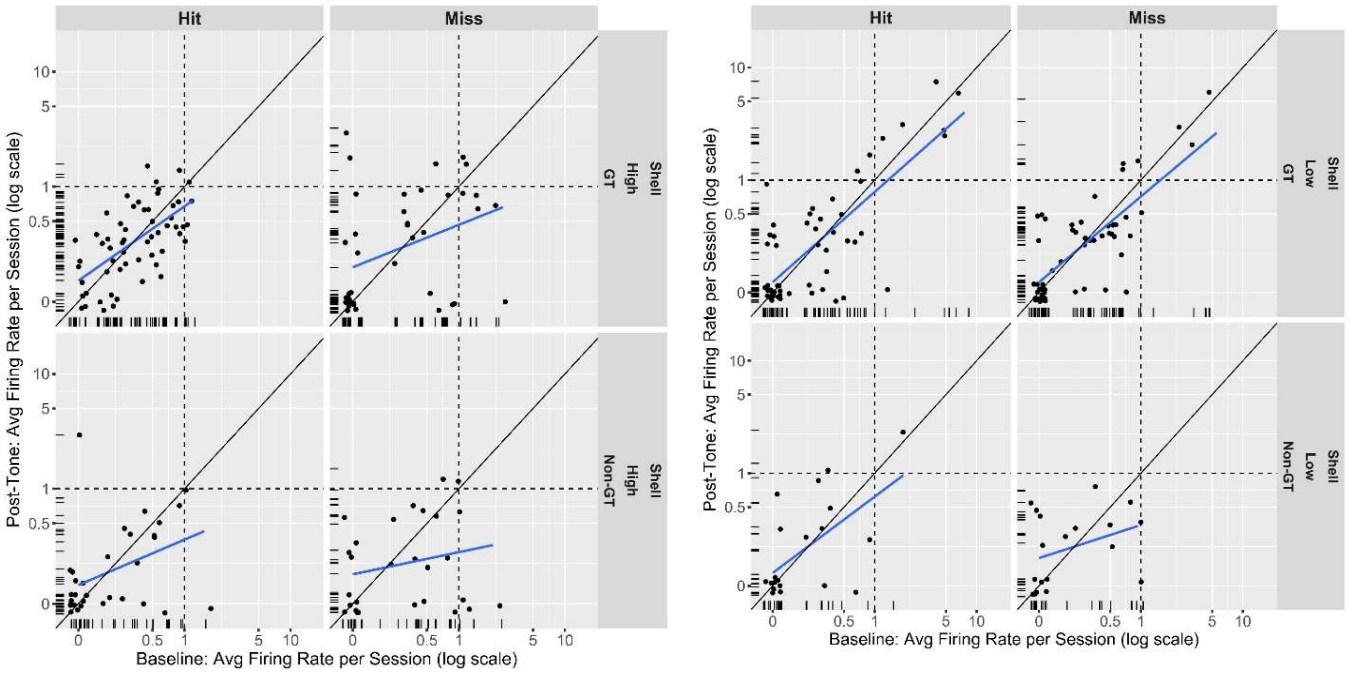

Figure 8b: Assessment of tone-evoked activity differences in Shell FR. What is the effect of baseline vs. posttone (TONE), are the FR different across Hits vs Misses (RESPONSE) and does this differ across GT v Non-GT (STGT)? For Shell neurons, there was no evidence of zero vs nonzero distributions Baseline vs Post, as the logistic model found significant main effects for STGT, $F(1, 544) = 5.19$, $p=.023$ but not for Tone, $F(1,544)=0.01$, $p>.10$ or Response, $F(1, 544) = 2.98$, $p>.05$. Significant two-way interactions were found for Response by STGT, $F(1, 544) = 9.37$, $p=.002$ and Response by Intake Category, $F(1, 544) = 8.48$, $p=.004$ but not for Tone by Response, $F(1, 544) = 0.68$, $p>.10$, Tone by STGT, $F(1, 544) = 0.00$, $p>.10$, Tone by Intake, $F(1, 544) = 0.78$, $p>.10$ or STGT by Intake, $F(1, 544) = 0.01$, $p>.10$ Significant three-way interactions were found for Response by STGT by Intake, $F(1, 544) = 12.28$, $p=.001$ but not for Tone by STGT by Intake, $F(1, 544) = 1.03$, $p>.10$ Tone by Response by STGT, $F(1, 544) = 0.02$, $p>.10$ or Tone by Response by Intake, $F(1, 544) = 0.61$, $p>.10$. The four-way interaction was not significant Tone by Response by STGT by Intake, $F(1, 544) = 0.00$, $p>.10$. This suggested no evidence of tone-evoked activity differences in GT or Non-GT, but there was evidence that neurons were generally silent or active on Hits or Misses (Response main effect). An analysis of this is shown in figure 9b.

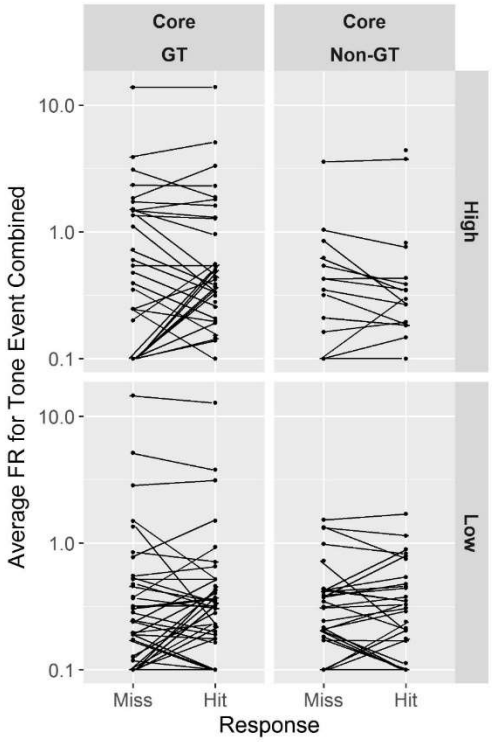

Figure 9A: Logistic Analysis of Core firing during Hit vs Miss trials: To understand the binary FR activity of Core neurons on Hits vs. Misses, we ran simple effects F-tests to analyze logistic FR differences of Response (Hit vs. Miss) for all possible combinations of STGT (GT vs. Non-GT) and Intake (High vs. Low).The distribution of 'Silent' (0 FR average for a session) vs 'Active' (Non-0 average session firing rate). The analysis revealed significantly more 'Active' neurons for Hit trials than Miss trials only for the GT-High Intake group, $F(1, 447) = 5.24$, $p. = .0226$. High intake Non-GT animals (and all low intake animals) showed similar distributions of logistic firing on hits and misses: Non-GT High, $F(1, 447) = 0.03$, $p. > .10$, Non-GT-Low intake $F(1, 447) = .78$, $p. > .10$, GT-Low Intake, $F(1, 447) = 1.33$, $p. > .10$. Therefore, a logistic pattern emerged in High Intake GT core neurons: silent on Misses but active on Hits (like their Shell activity in figure 9b)

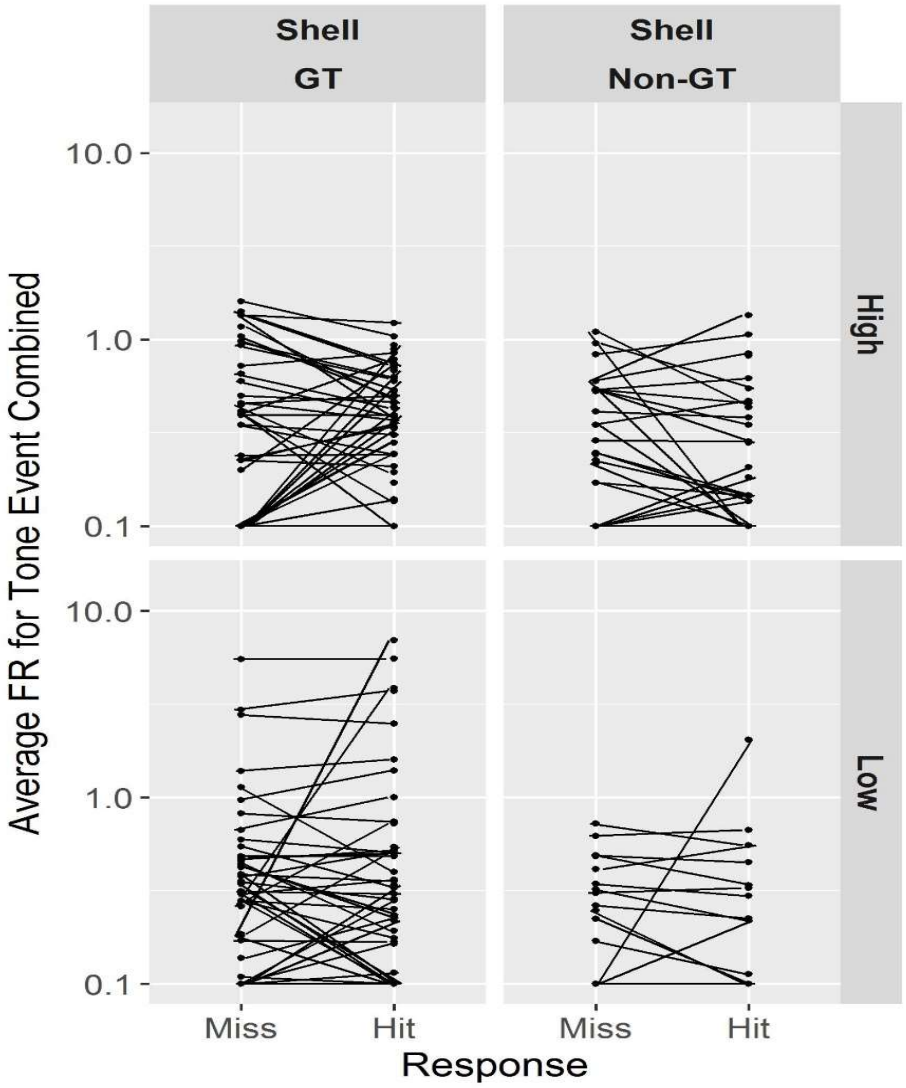

Figure 9b: Logistic Analysis of Shell firing during Hit vs Miss trials: A logistic analysis (zero nonzero) was run to analyze FR differences between Hits vs. Misses (Response) for all possible combinations of STGT and Intake. The distribution of 'silent' (0 FR for session) vs 'active' (Non-0 average session FR) revealed significantly more 'active' neurons for Hit trials, and 'silent' neurons for Miss trials in the GT-High Intake group, $F(1, 544) = 54.54$, $p. < .0001$. Non-GT animals (and all other subgroups) had a similar distribution of logistic firing on hits and misses Non-GT-High, $F(1, 544) = 1.17$, $p. > .10$, Non-GT-Low intake $F(1, 544) = .06$, $p. > .10$, GT-Low Intake, $F(1, 544) = 1.33$, $p. > .10$. Thus, although 'tone-evoked' activity was not present (NS Tone), high intake GT shell neurons were 'silent' on Miss trials across the entirety of a single session but became 'active' on Hit trials. This provides evidence that Nac activity may be associated with drug craving.

Region	STGT	Intake Category	Response	Pearson Correlation	Significance
1a. Core	Goal Tracker	High Intake	Hit	.77	<.0001****
			Miss	.71	<.0001****
		Low Intake	Hit	.69	<.0001****
			Miss	.85	<.0001****
	Non-Goal Tracker	High Intake	Hit	.67	0.0025**
			Miss	.80	0.0003***
		Low Intake	Hit	.82	<.0001****
			Miss	.58	<.0001****
1b. Shell	Goal Tracker	High Intake	Hit	.72	<.0001****
			Miss	.49	.0002***
		Low Intake	Hit	.78	<.0001****
			Miss	.77	<.0001****
	Non-Goal Tracker	High Intake	Hit	.39	0.0279*
			Miss	.22	0.2557 NS
		Low Intake	Hit	.51	.0043**
			Miss	.43	.0203*

For tables 1a and 1b: Neurons were recorded each day. The average FR for baseline (-200ms) and posttone (+200ms) were calculated independently to generate a 'neuron-session' for that specific recording day. Correlations were calculated between the baseline and posttone FR of that specific recording day to purposely restrict the analysis to that specific single neuron on the specific day. For example, the term "High intake, GT, Hit" refers to one day's recording for one neuron taken from an individual Goal-tracker animal and averages the spike rate for that single unit's 'hit' trials for Baseline and Posttone independently. All FR comparisons were restricted to compare the same neuron during different situations: Tone (Baseline versus Posttone) assessed the spiking-rate of the same neuron prior to the onset of cue (-200ms) and post (+200ms).

Table 1a: For Core neurons, stability between Baseline and Posttone FR was maintained for all conditions and significant positive correlations (Baseline vs Posttone) were found for all neuron-sessions (i.e., the avg neural response for each specified trial).

Table 1b: For Shell neurons, similar patterns of stability were shown for all combinations of STGT, Intake, and Response except for High intake Non-GT. Stability for this subgroup was significant during Hit trials but did not occur during Miss trials.

STGT	Animals with a High Intake Session	Total animals	High Intake Sessions	Total Sessions	%
GT	7	8	54	84	64%
Non-GT	4	5	23	47	49%

Table 2: High intake=consumed >69% of available drug (55 Hits). Non-GT= 4/5 animals with at least 1 high intake session, 23/47 sessions were high intake (49%) and GT=7/8 animals had at least 1 high intake session, 54/84 sessions were high intake (64%). A chi-square revealed no difference in distribution of 'high intake sessions' among GT and Non-GT $X^2(1, n = 131) = 0.087$, $p > .05$.