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DIFFERENTIAL NUCLEUS ACCUMBENS PROCESSING OF REWARD CUES IDENTIFIED IN "HIGH-RISK" VS. "LOW-RISK" GROUPS: IMPLICATIONS FOR SUBSTANCE USE DISORDER

By

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ABSTRACT OF THE DISSERTATION

DIFFERENTIAL NUCLEUS ACCUMBENS PROCESSING
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Dissertation Director:

Dr. Mark O. West

Not all individuals that use drugs will develop substance use disorder (SUD), and identification of individuals predisposed to develop SUD is critical for preventative health management of this major health crisis. SUD is driven primarily by negative affect and reinforced by compulsive drug use even after years of abstinence. This devastating relapse component of SUD is thought to be influenced by intense drug craving episodes that can be triggered by drug associated cues. Such drug cues have been shown to reinvigorate previous drug associations that have been established in the mesolimbic dopamine system. The mesolimbic dopamine system is involved in regulation of natural reward seeking behaviors, but cocaine "hijacks" the natural reward system by elevating dopamine in key limbic processing regions such as the Nucleus Accumbens (NAc), in the presence of cues associated with drug use. The NAc is a focus of cue-associated SUD research because of "limbic-motor-integration." The NAc shell subcomponent receives motivational signals from other limbic areas involved in rewarding activity. In turn, the

NAc core processes information from shell and projects to premotor areas to guide the final motivated action. Researchers interested in identification of individual differences in cue-predisposition have used Pavlovian autoshaping as a way to isolate distinctive phenotypes; 1) Goal-Trackers (GT) approached the foodport 2) Sign-Trackers (ST) approached and attacked the CS and 3) A third group of animals that were neither Sign nor Goal-Trackers but were regularly omitted (the present study defined these as "Non-Trackers", (NT)). ST were theorized to incentivize such reward cues, and have been studied as a phenotype predisposed to incentivize drug cues and have a higher likelihood of developing cue-induced relapse. Our experiment examined single NAc core and shell neuron changes in firing rate (FR) 200ms before and after the onset of a cue which signaled drug availability (tone-cue S^D). We studied whether this tone-evoked activity was different for the same neuron when the tone resulted in a drug seeking response (Hits) compared to interdigitated trials in which the tone did not result in a Hit (Misses). We also explored how different phenotypes (ST, GT, and NT), and Intake groups (High Intake (HI), and Low Intake (LI)) influenced self-administration behavior, tone-discrimination, and NAc tone-processing differences between Hits vs. Misses. Results demonstrate that phenotype did not influence drug consumption or behavioral tone-discrimination, and that HI subjects in general were not likely to behaviorally discriminate the tone relative to LI subjects of the same group. HI groups that were actually able to discriminate the tone behaviorally showed corresponding strong tone-evoked processing differences on Hits vs. Misses in NAc shell neurons. HI groups that did not discriminate the tone behaviorally did not show these strong differences in NAc

shell processing on Hits vs. Misses, a finding that unexpectedly was most prevalent among ST HI groups. Results do implicate ST as potentially a “high-risk” group with regard to cue-induced relapse, but other groups (GT, and NT) are at least as vulnerable to such risks and should always be included when studying phenotype differences in SUD.

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1. Introduction

1.0 Substance Use Disorder

Drug addiction is appropriately defined as substance use disorder (SUD). Hallmarks include compulsive drug seeking, drug use in spite of negative consequences, and long lasting changes to the brain and body (National Institute on Drug Abuse; National Institutes of Health; U.S. Department of Health and Human Services. 2018). The most recently updated diagnostic and statistical manual (DSM 5) defines SUD as a mental illness on a spectrum which ranges from mild, moderate, or severe based on a number of specific criteria. For example, such criteria include an inability to limit drug consumption, diminished effectiveness of the same quantity of drug, increased drug use to achieve the desired effect, and drug use to relieve withdrawal symptoms rather than or in addition to produce euphoria. (National Institute on Drug Abuse; National Institutes of Health; U.S. Department of Health and Human Services. 2018). It is estimated that over 21.5 million Americans suffer from SUD (Center for Behavioral Health Statistics and Quality, 2015) and the CDC reported 72,237 deaths due to drug overdose in 2017 alone (Hedegaard *et al.,* 2018). It is clear that SUD is a major health concern of the United States and around the world.

Humans suffering with SUD often report a snowballing loss of control over drug intake (Hammer *et al.,* 2012) and that, over time, it becomes impossible to achieve the same initial 'pleasurable' drug experience (Baker *et al.,* 2004). Animal models of SUD suggest a similar phenomenon to the human experience. Animal subjects regularly

acquire drug self-administration (SA) when given the opportunity to use drugs (Coffey *et al.,* 2015) and escalate drug consumption over days when given repeated long access to drug (Ahmed & Koob, 1998). Over time, this escalation leads to individual differences in "preferred" drug level (DL) (Root *et al.,* 2009; Root *et al.,* 2011) which is reflected in humans suffering from SUD (Kelly *et al.,* 2006). In animal models of SUD, this preferred DL is maintained through a process known as "titration", which includes ignoring opportunities to self-infuse when DL is too high, but responding when internal DL is low (Pickens, Thompson & Yokel 1972), in a manner consistent with negative reinforcement that delays inevitable drug withdrawal (Zimmer *et al.*, 2013; Barker *et al.*, 2014). This typical SA pattern in animals also mirrors human reports that the daily perils of drug addiction involve negative, rather than positive, reinforcement.

1.1 Effect of Cocaine on Anatomical and Neurophysiological Systems

An undeniable reality of SUD is that, without intervention, chronic drug abuse will continually bombard the same physiological systems in the body and brain and lead to permanent changes and/or damage. Such physiological impacts resulting from chronic drug-abuse are well documented in humans and animals. For example, cocaine is a stimulant drug which induces short term euphoria associated with elevated heart rate, energy, and hypersensitive environmental awareness (National Institute on Drug Abuse; National Institutes of Health; U.S. Department of Health and Human Services). Neurophysiologically, short term cocaine use acts by disrupting typical neuronal reuptake of dopamine, serotonin, and noradrenaline and "trapping" supranormal quantities of neurotransmitter between neurons within the brain's motivational regions,

such as the mesolimbic dopamine system (Ritz *et al.,* 1990). However, repeated drug use can result in permanent damage to the heart and other organs (National Institute on Drug Abuse; National Institutes of Health; U.S. Department of Health and Human Services. 2018) as well as maladaptive plastic adaptations in the aforementioned brain systems.

The mesolimbic dopamine system has long been associated with regulation of goal-oriented behavior, such as food and sexual seeking (Martel & Fantino, 1996) and it is particularly activated by drugs of abuse. This system is critical in natural reward learning, in which natural or drug rewards excite dopaminergic transmission from ventral tegmental area (VTA) to the nucleus accumbens (NAc), prefrontal cortex (PFC), and amygdala (Kelley & Berridge, 2002). This coincides with other neurotransmitter activity; the NAc projects GABAergic signals directly to VTA, and indirectly via the hypothalamus and ventral pallidum. Convergent glutamatergic signals regarding available cues project from PFC and amygdala to NAc and VTA (Kelley & Berridge, 2002). It has been hypothesized that these intra-limbic signals spiral laterally throughout the system and eventually project to premotor areas which guide the intended goal-directed motor response (Haber *et al.*, 2000).

Dopaminergic transmission is particularly critical for the rewarding effect from both natural and drug rewards (Wise, 1983). In fact, several of these aforementioned dopamine specific regions respond directly to natural and drug rewards; VTA following sucrose reward (Schultz *et al.,* 1993) and cocaine self-administration (SA) (Einhorn *et al.,* 1988), the PFC during ejaculation (Georgiadis *et al.,* 2007) and cocaine SA (Chang *et al.,*

1997;1998), the Amygdala during sucrose administration (Tye *et al.,* 2010) and cocaine SA (Hurd *et al.,* 1997), and the NAc during water reinforcement (Carelli & Deadwyler 1994; Young *et al.,* 1992) as well as cocaine SA (Peoples *et al.,* 1993; 1998; 1999, Uzwiak *et al.,* 1997) and ethanol SA (Janak *et al.,* 1999).

Not surprisingly, imbalances within the dopaminergic system dramatically influence predisposition for addictive tendencies among individuals (Grimm *et al.,* 2003). Essentially, cocaine (and other drugs of abuse) manage to "hijack" this important natural reward system by supranormally elevating concentrations of DA in key aforementioned regions such as the NAc at dramatically higher volume than natural rewards (Willuhn *et al.*, 2014). The NAc is considered a critical relay hub involved in gating/forwarding limbic signals to motor areas of cortex and influencing motivated actions. Anatomically, the shell is considered 'upstream' from core and receives rich limbic inputs from the amygdala, ventral subiculum, VTA, and limbic cortical processing regions (Chesselet *et al*., 1998; Parkinson *et al.,* 1999, Pockros *et al.,* 2011). The core is "downstream" from the shell (Haber *et al.*, 2000), and is striatal-like, with projections to premotor areas which influence movements through proposed laterally spiraling striatal connections. Therefore, due to this motivational-striatal-motor relationship, the NAc has been targeted frequently to explore the motivational mechanisms underlying drug-seeking behavior, addiction, and cue-induced relapse (Ghitza *et al.,* 2003; 2004; Lin & Pratt 2014; Cui, Thakkar, Sullivan, *et al.,* 2015).

1.2 Cues Influence SUD and Neurophysiological Systems

One explanation for the complicated nature of chronic drug-relapse is that, despite the myriad of negative consequences, a multitude of local and environmental cues become associated with previous drug abuse and can act as a triggers for intense drug-craving episodes (Volkow *et al*., 2008). In fact, environmental drug-cues can trigger persistent drug seeking and relapse in humans (Ehrman *et al.,* 1992) and in animal models (Waters *et al*., 2014), in spite of negative consequences (Shaham & Zangen *et al.,* 2007), even in the absence of the drug itself (See *et al.,* 2003). Processing of cues is a critical early stage of "limbic-motor integration" (Nauta *et al.*, 1978) consistent with laterally spiraling circuitry (Haber *et al.*, 2000).

Indeed, studies have demonstrated that cues associated with natural and drug rewards activate the dopamine system. For natural rewards, cue presentation induces activity in limbic regions that project to the NAc such as 1) the VTA during sexual cue presentation (Balfour *et al.,* 2004), and 2) both the amygdala (Nishijo *et al.,* 2003) and PFC (Matsumoto *et al.,* 2003) during sucrose cue presentation. The NAc also is directly responsive to natural reward cues. For example, during sucrose tasks, single NAc neurons change in firing rate (FR) in response to discriminative stimuli (S^D), i.e., cues which signal availability of reward contingent upon a response (Nicola *et al.,* 2004). Further, selective antagonism of NAc dopamine disrupts behavioral responses to food predictive cues (Yun *et al.,* 2004). Finally, both NAc core and shell neurons respond to sexual cue presentation (Balfour *et al.,* 2004).

Similarly, the influence of environmental or contextual drug cues re-invigorates drug associations within the mesolimbic dopamine system (Ikemoto & Wise, 2004; Thomas,

Kalivas & Shaham 2008). Such drug associated cues activate regions that project to the NAc, such as the VTA and Amygdala during cocaine cue presentation (Wise, 2009; Meil & See, 1997; Weiss *et al.,* 2000) and presentation of alcohol cues result in strong PFC activity among humans suffering from alcoholism (Grüsser *et al.,* 2004). Drug cue presentation also changes NAc core activity (Hollander & Carelli 2005) and presentation of a cue formerly associated with cocaine resulted in strong NAc shell FR changes during extinction trials (Ghitza *et al.,* 2003). However, it was unclear whether the change in neural activity was related to new learning of extinction mechanisms, or due to reinvigoration of previously strong drug-cue associations. Indeed, a third possibility is a pharmacological influence, because the same neurons exhibited little cue-evoked activity during cocaine SA (Root et al, unpublished, preliminary observations). Therefore, a major goal of this study was to identify whether changes in NAc core and shell FR occur in response to a cocaine-specific S^D during cocaine SA instead of during extinction.

1.3 Rationale

Researchers interested in individual differences in reward cue-predisposition have used Pavlovian autoshaping, a classical conditioning paradigm which involves the presentation of a retractable “lever” as a conditioned stimulus (CS) followed by the automatic delivery of an unconditioned stimulus (US) sucrose pellet reward into the foodport (Robinson & Berridge, 1993). Pavlovian autoshaping is alternatively known as “Sign/Goal-Tracking”, and has been used as a way to isolate distinctive cue-reactive phenotypes; 1) Goal-Trackers (GT), were subjects that approached and interacted with the foodport 2) Sign-Trackers (ST), approached and attacked the CS and 3) A third group

of animals that were neither Sign, nor Goal-Trackers and were regularly omitted (the present study defined these as “Non-Trackers”, (NT)). ST were theorized to incentivize such reward cues, and have been studied as a phenotype predisposed to incentivize drug cues and have a higher likelihood of developing cue-induced relapse (Piazza *et al.*, 2000; Flagel *et al.,* 2007; Robinson & Flagel, 2009; Flagel *et al.*, 2010; Saunders, Yager & Robinson, 2013).

With STGT phenotypes translatable to humans (Garofalo & di Pellegrino, 2015) and drug addiction known to affect people of all backgrounds (Grant *et al*., 2006), it is likely that individual STGT differences may enable heterogeneous neurobehavioral formations of addiction. Therefore, the aim of this experiment was to study drug-taking behaviors across 15 days of SA among rats identified as Sign-Trackers (ST), Goal-Trackers (GT), or Neither-Trackers (NT) based on a continuum of STGT criteria observed during a separate STGT pretest. Specifically, we tested whether STGT phenotype influenced overall drug consumption during SA, behavioral discrimination of drug cues during drug-free periods, escalation of drug intake, and NAc processing of drug-related cues during the session.

Based on literature suggesting that drug intake is similar between ST and GT (Tunstall & Kearns 2015; Bardo *et al.*, 2006) we expected that all three groups would self-administer similar amounts of drug across the entirety of the SA sessions, and that STGT phenotype would not influence any aspect of cocaine consumption.

However, there are published reports which suggest certain behaviors differ between ST and GT groups. For example, ST reduce drug-seeking behavior when

previously paired drug-cues were removed during SA (Saunders & Robinson, 2010; 2013) and ST are theorized to be hypersensitive to drug associated stimuli compared to GT. Therefore, we predicted that ST would not only acquire the discriminative properties of the tone over the course of SA, but also demonstrate a greater ability to avoid responding in the operant corner during periods of drug unavailability (tone S^D off) compared to GT and NT. Specifically, we predicted ST would show significantly lower rates of uncued responding during the SA session compared to GT and NT. Furthermore, because of expected strong ST association of external cues we anticipated that ST would titrate internal DL in response to drug availability signaled by the tone-cue S^D. We also predicted that this would manifest in lower fluctuations of DL within session compared to HI and LI GT and NT, with ST predicted to show lower Hit DL relative to higher Miss DL (i.e., titration).

An additional component of the present design was to study the influence of different *preferred* DL on behavior and NAc processing of the tone S^D. High intake (HI) groups could be considered “high risk” because elevated drug intake is associated with increased risk of addiction development (Wolffgramm & Hyene 1995). We planned to evaluate HI groups independently of low intake (LI) groups as a way to identify if differences were unique to “high risk" GT, NT, or ST.

Importantly, our repeated 6 hour cocaine SA sessions utilized intermittent drug-availability signaled by a specific tone-cue S^D. The presentation of the cue at variable intervals enabled animals to choose to take drug (“Hit”) or avoid opportunities to self-administer (“Miss”) on each trial. We were particularly interested in how the same NAc

neuron processed the tone-cue S^D during Hits (i.e., when the cue actually triggered drug seeking) vs. interdigitated trials on which the cue evoked no response during Misses.

The comparison between Hits and Misses was a major component of the design as NAc core and shell neurons were hypothesized to process the same tone-cue S^D differently across the two different motivational states. With regard to NAc core and shell firing, it was possible that different phenotypes (GT, NT, ST) and DL preference (HI, LI) could enable individual differences in drug cue sensitivity. Specifically, we predicted that ST would exhibit NAc related FR changes in response to the tone-cue S^D, and predicted tone-evoked changes among ST for both Hit and Miss trials during SA in core and shell neurons. We also predicted that this would be strongest among HI ST relative to LI ST and all other subgroups. Implications for SUD could be increased risk of cue-induced drug relapse in “high risk” humans defined as ST, GT, or NT.

2. Materials and Methods:

2.1—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 (SA) paradigm on a 12/12 light/dark cycle, with light onset at 10:30 am. Animals' weights were maintained at ~350g with water and food rations provided to maintain this weight following the end of all experimental sessions (both the STGT pretest and cocaine SA). All animal conditions were maintained in compliance with the Guide for the Care and Use of Laboratory Animals (NIH, Publications 865–23) and approved by the Institutional Animal Care and Use Committee, Rutgers University.

2.2—Sign and Goal Tracking: STGT

2.2.1—STGT: Pretest

Prior to cocaine self-administration all subjects (n=139) were pretested using a traditional STGT paradigm to determine individual phenotype across seven days. All subjects were food deprived the morning of each pretest session and given food rations upon completion of the session.

1) Pretraining (2 sessions): Consistent with Flagel *et al.* (2007), animals were first given two pretraining sessions. For each pretraining session 50 sucrose pellets were automatically dispensed on a 90s variable time schedule over 25 minutes while the lever

remained absent. The foodport was examined following each pretraining session to confirm that all pellets were consumed.

2a) Training (5 sessions): Each training session comprised 25 trials, and consisted of the presentation of a retractable lever (conditioned stimulus, **CS**) followed by a sucrose pellet automatically dispensed into the foodport eight seconds later (unconditioned stimulus, **US**) whereby the lever immediately retracted into the slot. Following reward delivery, a variable 30-90 second ITI occurred prior to the next trial.

2b) Recording During Training: All lever interactions (i.e., number of lever presses, **LP**) caused by pushing, gnawing, or manipulating the lever were recorded with no programmed consequences. In addition, the total number of foodport (**FP**) entries, and the average latency to enter the FP following lever presentation (**Latency**) were recorded as additional parameters for STGT designation based on preliminary data for non-ST animals and from similar procedures in the literature (Pitchers *et. al.* 2017).

2.2.2—STGT: Analysis and Identification of ST, GT, and NT groups

*Pretest Leverpressing as an identifier of phenotype***:** Low LP has been associated with Goal-Tracking (Robinson *et al.* 1993). A histogram of all subject pretest LP was generated and phenotype was identified by selecting the lowest 33rd percentile LP (GT), the middle 33rd percentile LP (NT), and highest 33rd percentile (ST) (Fig. 1).

Confirmation of LP as a definitive measure of Phenotype, Composite Score: To confirm LP as an appropriate measure of Goal or Sign tracking, an additional **Composite Score** was created using the three additional measures obtained during the pretest: LP, FP, and

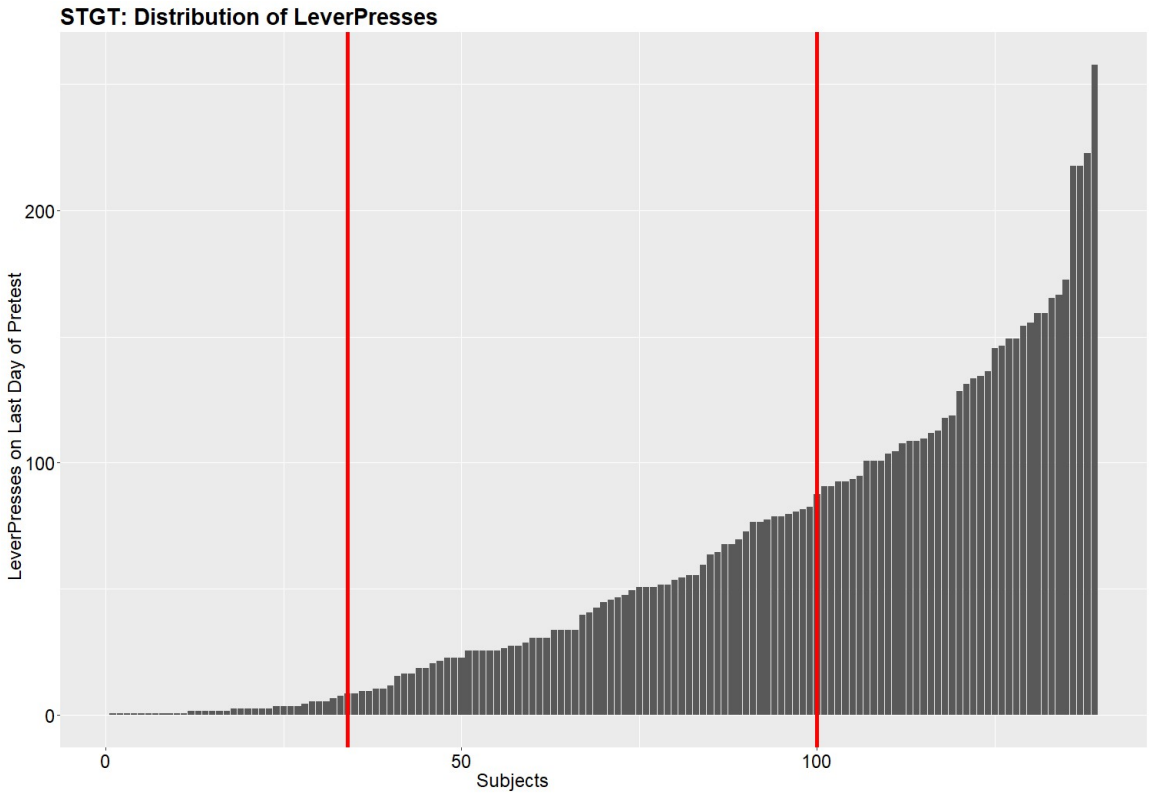

Figure 1: Distribution of animal "LeverPresses" during the final day of the STGT pretest. The y axis represents the number of LeverPresses during the final day of the STGT pretest and the x axis is the number of subjects (binsize=1). The vertical intercepts represent the demarcations for the lowest 33% of Leverpressers (Goal-Trackers), the middle 34% (Non-Trackers), and the highest 33% (Sign-Trackers).

Latency. All animals were given a score of 1-3 for each of the three categories, where a score of 3 for each category represented strong GT traits and all scores were then summed to a maximum score of 9. For example, a "strong GT" would be associated with Low LP, High FP, and Low Latency (and result in a score close to 9). It was predicted that a high Composite score would be correlated with a low number of LP (i.e., strong GT). A linear regression analysis was performed using R-Studio (R Core Team, 2014) using the packages Lsmeans and Psych and graphics were produced using the package ggplot2 (Wickham, 2009). Composite Score was specified as the DV and was regressed on LP during the final day of the pretest.

2.3—Cocaine Self-Administration

2.3.1—Surgery: Catheterization and Microwire Implantation

For detailed surgical description of catheterization and Microwire implantation, see (Root *et al.,* 2013; Barker *et al*., 2014). Following the pretest selected subjects (n=27) were permanently implanted with an intravenous jugular catheter, and a 2x8 stainless steel microwire array (MicroProbes for Life Science, Gaithersburg, MD) was lowered into the right NAc core and shell according to Paxinos & Watson Rat Brain in Stereotaxic Coordinates atlas (1997) in order to perform single unit recordings. Localization of wires (i.e., core, shell, or other) were identified with histological calbindin immunohistochemical staining following completion of the experiment.

2.3.2—Surgical Recovery and Self-Administration Apparatus

Post-operative animals recovered for one week in customized Plexiglas SA chambers (30 x 25 x 25 cm) and remained single housed in their own SA chamber for the duration of the experiment. Each chamber included a corner with a customized 6-photocell device permanently fixated outside of the Plexiglas to record operant "nosepoke" responses (Root *et al.,* 2011). The operant corner remained constantly available to facilitate acquisition of the S^D properties of the tone during daily 6-hour SA sessions. Animal catheter lines retained patency during recovery, and overnight post-experiment, via infusions of .2ml of heparinized bacteriostatic saline every 25 minutes.

2.3.3—Cocaine Self-Administration Paradigm

Cocaine sessions consisted of 6 hour daily SA for 14 consecutive days followed by a three day abstinence period and a final 15^{th} day of SA. For the purpose of this study, the abstinence sessions were not studied because the tone was absent. The 15^{th} session was treated as a continuation of the SA period, not as a separate measure of analysis. The first three days of SA (1-3) were considered "**Early**" sessions and the remaining sessions (4-15) were considered "**Late**".

Before every SA session, prior to the light onset of the 12/12 light/dark cycle, any remaining overnight food/water was removed, subject body weight was recorded (in grams), and the animal was 'plugged' into their recording harness at the headstage. SA sessions began immediately at light-onset of the light/dark cycle. For each trial, cocaine availability was signaled by the onset of a 30 sec 3.5 kHz tone-cue, i.e., discriminative stimulus, S^D. A "nose-poke" response in the operant corner (breaking photocell #2) at

any time during the 30 sec tone immediately halted the tone and automatically activated the cocaine pump which administered a 0.7 mg/kg infusion of cocaine (0.24mg/0.2mL) over 7.5s into the catheter. Every trial was designated with respect to **Response**; any trial in which the animal self-administered drug by responding during the 30 sec tone was defined as a "**Hit**", or cued drug seeking response. Any trial in which the tone did not evoke a Hit was defined as a "**Miss**," i.e., the animal "missed" the opportunity to self-administer drug. All tones leading to the first Hit of each session were defined as the **Predrug phase.** Until the 10th Hit of each session (the first 10 hits were defined as the **Loadup phase**)**,** tone-offsets were followed by a 40s "tone-off" intertrial interval (ITI). Following the 10^{th} Hit, the ITI shifted to a pseudo-random 1-6 min ITI for the remainder of the 6-hour session (approximately 5 to 5.5 hours), and was defined as the **Maintenance phase.** Nosepokes that broke photocell #2 during the ITI for any phase did not result in cocaine delivery or any programmed consequences, but were recorded as individual uncued nosepokes which were analyzed in finer detail in 2.3.5.

2.3.4—Drug Level Calculation

Drug Level (**DL**) in uMole brain level of cocaine was calculated in real time at a 1 second resolution during the session, consistent with Pan *et al.* (1991) using the following equation:

$$DL = \left(\frac{d*k}{v(\alpha-\beta)}\right)\left(e^{-\beta*t} - e^{-\alpha*t}\right) \quad (1)$$

where d = drug dosage (mg/kg), k = 0.233/min (rate of flow between two compartments, v = 0.15 L/kg (brain volume), α = 0.642 min & β = 0.097 min (constants

where α represent the redistribution, β represents the conversion/elimination of cocaine and metabolites), and t = time (in minutes) since the last infusion. **Equation 1** was consistent with Lau & Sun (2002).

2.3.5—Uncued Responding: Uncued Nosepokes represent a "Flutter", rather than individual Responses

Detection of "UR-flutter": Analysis indicated that individual uncued nosepokes occurred in bursts. Rather than emitting a single nosepoke, rats moved their head up and down rapidly in the corner, and broke the photocell multiple times on each visit to the operant corner (i.e., a "flutter" of nosepokes). Specifically, on Hits, the first vertical head movement through the photocell registered as the Hit response, followed by a flurry of continued vertical head movements (flutter) which all registered as uncued nosepokes. We also observed this flutter during the ITI (uncued, independent of Hits) in a similar distribution. It was not clear if the nosepokes during the ITI were artifacts associated with stimulant-induced stereotypy or if these nosepokes were representative of drug seeking during periods of drug unavailability, and potentially compulsive drug seeking.

Identification of distinct response clusters of both Cued and Uncued response topography: To better understand these patterns we first obtained a measure of 'Uncued Responses (**UR**) for two categories: nosepoke flutter after a Hit (**Cued, Hit:UR intervals**) and after an uncued nosepoke (**Uncued, UR:UR intervals**). We then used a behavioral analysis consistent with that of Zimmer & Roberts (2013):

Uncued UR:UR intervals were defined as the difference (in seconds) between consecutive uncued nosepokes, where each distinct UR created a node for graphing the latency to the next UR. **Cued Hit:UR intervals** were calculated a similar way, where each distinct Cued Hit created a node for graphing the latency to the first UR following a Hit. Inter-response interval histograms were generated during all Late sessions for the Maintenance phase of SA (Fig. 2). Inspection of histograms revealed intervals within 2s contained the highest proportion of the data for both Cued and Uncued intervals. Therefore, individual nosepoke photocell breaks within 2s of each other (i.e., within the same flutter) were considered part of one continuous response rather than as separate drug seeking responses. For all subsequent analyses, UR:UR intervals <2s were considered part of the same **Uncued Response (UR)**. Uncued Responses were recorded for every session and were a main unit of observation for several subsequent behavioral analyses.

Uncued Nosepokes Centered on Hit as the Node: A graphical analysis was also performed using the onset of the tone on a "Hit" as a node during Maintenance phase of all late sessions for "Titrate" and "NoTitrate", HI and LI, ST and NT and GT groups. The purpose of this analysis was to identify if the onset of the tone resulted in a change in responding for any aforementioned category. I.e., do animals continuously nosepoke until the tone comes on? How does the last UR before the onset of the tone on a Hit compare to the first UR immediately after the onset of the tone? Histograms were generated for counts of intervals (in .2 seconds bins) for the last UR prior to the tone (negative value) vs. immediately following the tone (positive value).

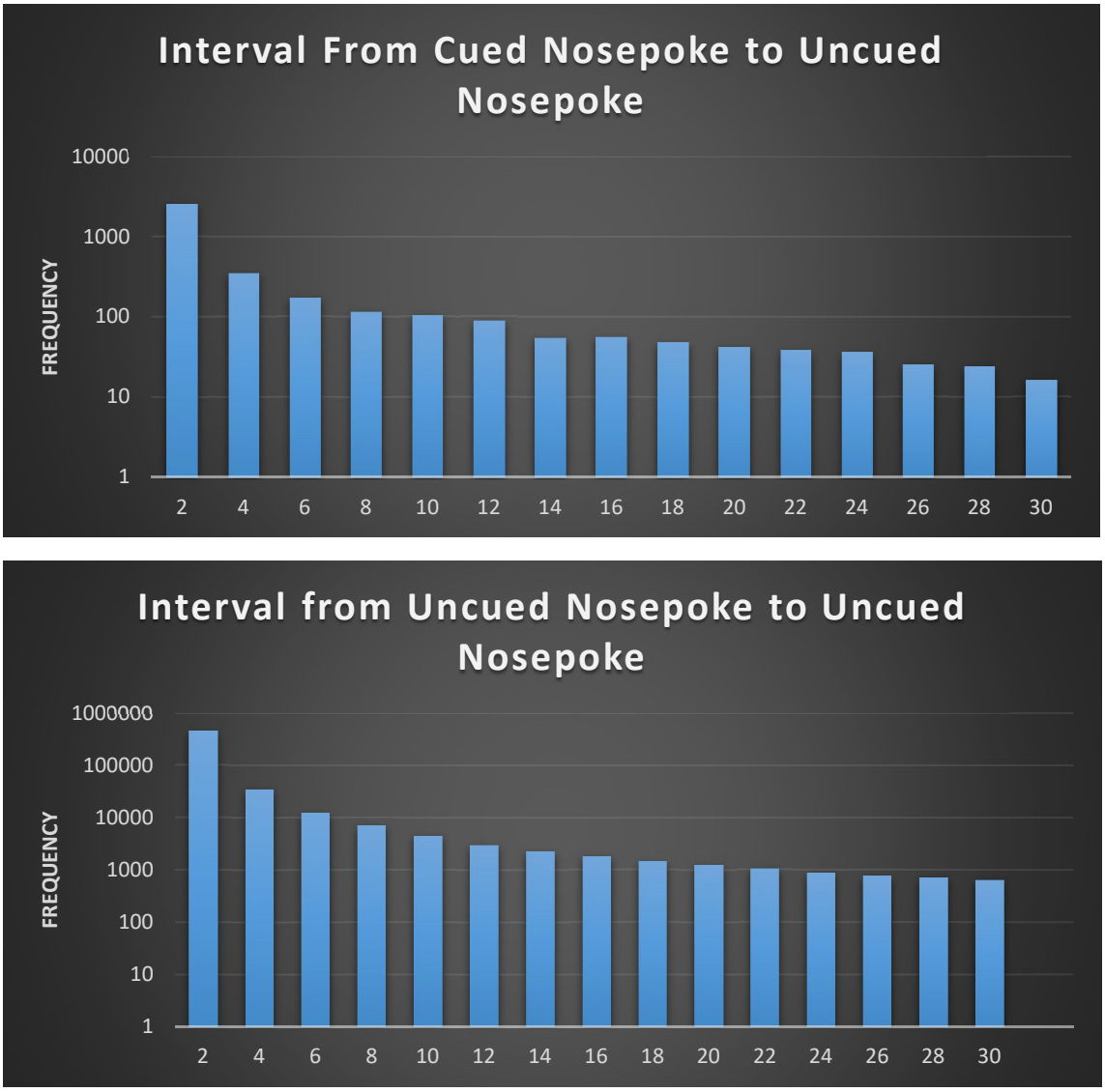

Figure 2: Analysis of "Flutter" of Nosepoke Intervals: Hit to first uncued response (top) and Uncued Response to the next Uncued Response (bottom) presented in 2 second bins. The y axis is log scaled for both figures, and the distributions of Hit:Uncued and Uncued:Uncued response intervals were similar, and intervals within 2s (the first column) contained the highest proportion of the data for both figures (Cued=69% of all data, Uncued=86% of all data). Therefore, a cutpoint of 2s was chosen as the line of demarcation for what would be considered a unique response. In addition, "Uncued Responses" made during the ITI were not randomized stereotypy, but rather true uncued drug responding during periods of drug unavailability.

3.0—Specific Behavioral Calculations and Analyses for Specific SA Phases

Consistent with Coffey *et al.* (2015), individual sessions were subdivided into distinct phases for specific analyses:

3.1--Predrug Phase:

After 18 hours' withdrawal, cocaine levels were negligible or nonexistent so all tones leading to the first Hit of the session were defined as the Predrug phase. All Predrug trials utilized the same 30 second tone cue S^D and a fixed 40 second ITI.

3.1--Predrug Phase: Calculations

*Predrug Response Rate*s: It was vital to identify if all subjects acquired tone discrimination over the course of two weeks of cocaine SA. Acquisition of selective tone-discrimination was determined by first identifying response rate during tone-off periods "**Uncued Predrug Response Rate**" (**equation 2**) vs. responses during tone-on periods "**Cued Predrug Response Rate**" (**equation 3**).

$$Uncued\ Predrug\ Response\ Rate = \left(\frac{Number\ of\ URs}{Predrug\ Total\ Summed\ ITI\ Time}\right) \quad (2)$$

Equation 2: The numerator was total Predrug URs and the denominator is total time (in seconds) the tone was off, and drug was unavailable, over all Predrug trials.

$$Cued\ Predrug\ Response\ Rate = \left(\frac{1}{Pre{-}Drug\ Total\ Summed\ Tone\ \ Time}\right) \quad (3)$$

Equation 3: The 1 in the numerator is the first Hit of the session and the denominator is total time (in seconds) the tone was on, and drug was available, prior to the first Hit. This also includes the latency (in seconds) to respond to the tone on the first Hit trial.

3.1.1--Predrug Phase: **Planned Analyses**

The purpose of this study was to identify NAc processing of the tone-cue S^D, and it was vital to determine if animals acquired the tone and task at all, and if this differed between GT, NT and ST. Acquisition of tone-discrimination was assessed using a repeated measures ANOVA model in R where Uncued Predrug Response Rate was compared to Cued Predrug Response Rate across **Early** vs. **Late** sessions. Both **Predrug Response Rates** (**Cued** and **Uncued)** were included in the DV, while **Sessions** ("**Early**" or "**Late**") and **STNTGT** (GT NT and ST) were categorical IVs.

3.2—Loadup Phase:

Following the first Hit, all trials leading to each session's first 10 Hits used identical operant conditioning parameters as the Predrug phase (i.e., 30 second tone-on and 40 second ITI) and was defined as Loadup. The Loadup phase was not a point of interest in this study and was excluded from further analyses. Instead, the Loadup phase allowed animals an opportunity to self-administer drug to achieve their preferred maintenance level before the randomized ITI was enabled during Maintenance.

3.3—Maintenance Phase:

For the present study, the Maintenance phase contained the majority of behavioral data, and all neural data. Maintenance was defined as all trials following Loadup until the end of the session, i.e., Hits 11 through 80 or the end of 6 Hours, whichever occurred first.

3.3—Maintenance Phase: Calculations

Pseudo-Randomized Intertrial Intervals: Maintenance utilized the same operant parameters associated with the 30 second tone-cue S^D, but the 40 second ITI was replaced with a pseudo-randomized variable intertrial interval of 1-6 minutes. The purpose was to enable the animal to experience both being above "satiety" on ~50% of the trials (e.g., short intervals, when drug level remained high, after a recent infusion), as well as being below satiety on the other ~50% of trials (e.g., long intervals, after pharmacokinetic decay of drug level). This is represented in Figure 3. The variable interval schedule accomplished two purposes. First, we wanted to compare NAc FR patterns from the same neuron on Hit trials (assuming that the tone was highly salient and motivational) compared to Miss trials (assuming that the tone was not salient

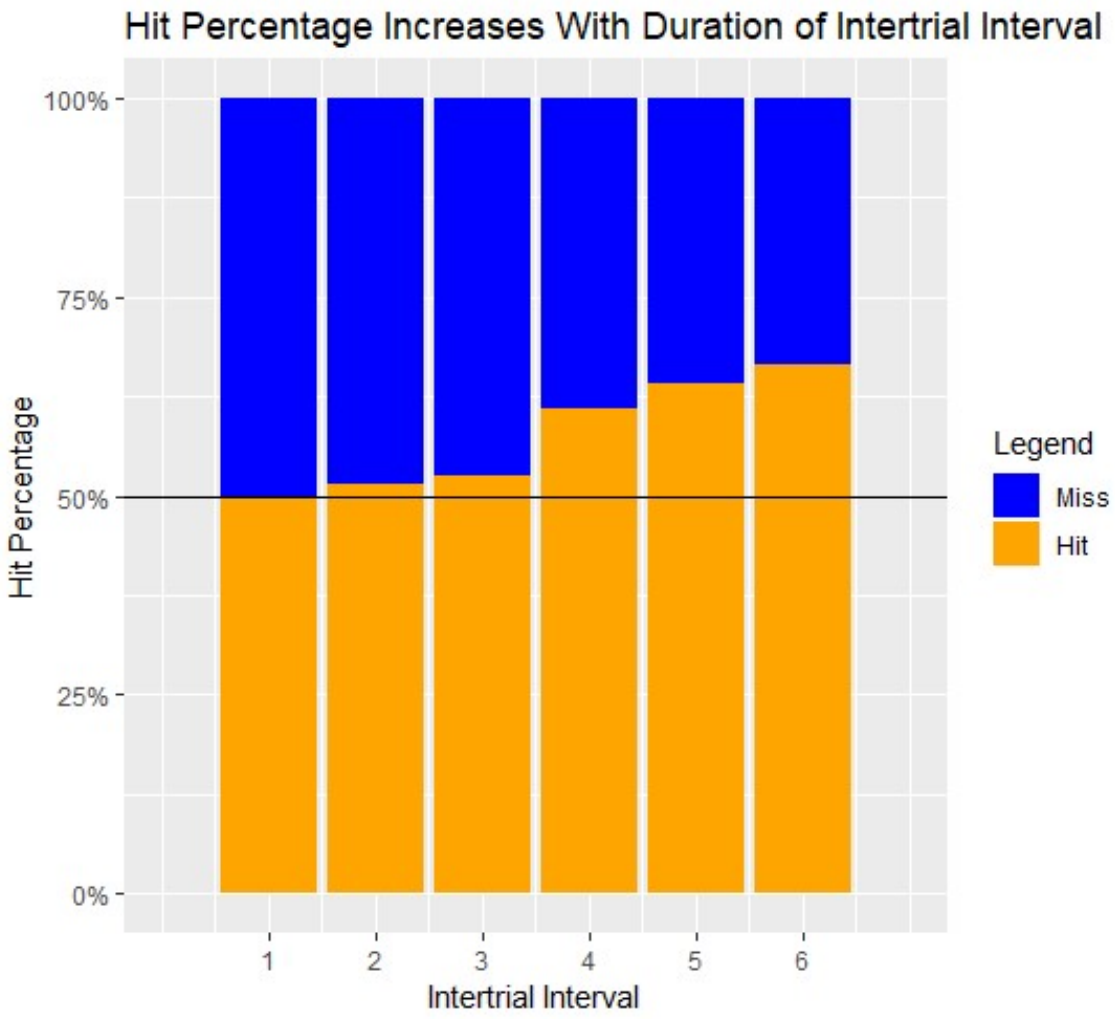

Figure 3: Hit percentage increases with drug level. These data represent all intertrial intervals from all late sessions during maintenance grouped in 1 minute bins. The color of the bar refers to the percent of the trials that resulted in a Hit response (orange) compared to a Miss response (blue). Data suggest that a long duration ITI (>3 minutes) resulted in a greater likelihood of a Hit response while shorter intertrial intervals resulted in approximately 50% Hits and 50% Misses.

enough to prompt a self-infusion). Second, animals could learn that they were able to achieve drug satiety in every session, which avoided the potential for frustration in other schedules in which infusions occur too infrequently for animals to raise their drug level to their preferred, or "satiety" levels.

High Intake Identification: Despite the inter-trial interval planning, it was still possible that individuals could be below satiety during a session. This potential issue was especially important because High intake groups could be considered "high risk" as heightened drug intake is associated with increased risk of addiction development (Wolffgramm & Hyene 1995). Therefore, for purposes of analysis, a cutpoint was derived from the median drug consumption across all animals, calculated during Late Maintenance sessions (X=8.23 mg/kg). Total number of Hits was multiplied by the dose of cocaine per infusion divided by subject body weight (kg). The purpose of this cutpoint was to evaluate the relationship between NAc FR and behavioral data, independently within High Intake sessions (**HI**, mean drug consumption>8.23mg/kg) and within Low Intake sessions (**LI**, mean drug consumption<8.23mg/kg), but not to compare between Intake categories.

Analysis of "Titration": Numerous reports indicate that animals self-administer cocaine to maintain 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 *et al.,* 2009; Root *et al.,* 2011; Zimmer *et al.,* 2013). This is otherwise known as "titration", and was calculated for each session by subtracting the average drug level during Hit trials from that during Miss

trials throughout the maintenance phase to create a drug level difference score (DLDIFF). **DLDIFF** was calculated as:

$$Drug\ Level\ Difference\ (DLDIFF) = MissDL - HitDL \quad (4)$$

where average DL on **Hits** (HitDL) and **Misses** (MissDL) were from the same session and animal. When DLDIFF was positive, DL was higher on Miss trials than Hit trials during the session (**higher-expected, or "Titrate")** and was considered sufficient evidence of titration. Negative DLDIFF values (**lower-unexpected, "NoTitrate"**) were therefore evidence of lack of titration or possibly inability to titrate.

*Maintenance Response Rate*s: It was vital to identify if all subjects continued to discriminate the tone during the Maintenance phase of the session. We calculated "**Uncued Maintenance Response Rate**" (**equation 5**) vs. responses during tone-on periods "**Cued Maintenance Response Rate**" (**equation 6**) similarly to the predrug response rates.

$$Uncued\ Maintenance\ Response\ Rate = \left(\frac{Number\ of\ URs}{Maintenance\ Total\ Summed\ ITI\ Time}\right) \quad (5)$$

Equation 5: The numerator was total Maintenance URs and the denominator was total time (in seconds) the tone was off, and drug was unavailable, over all Maintenance trials.

$$Cued\ Maintenance\ Response\ Rate = \left(\frac{Number\ of\ Hits}{Maintenance\ Total\ Summed\ Tone\ \ Time}\right) \quad (6)$$

Equation 6: The numerator refers to the number of self-infusions achieved during Maintenance and the denominator is total time (in seconds) the tone was on, and drug was available, during Maintenance. This included the sum of all latencies (in seconds) to respond to the tone over all Hits trials plus the sum of tone-onset across all Miss trials.

3.3.0—Maintenance Phase: Planned Analysis

The purpose of this experiment was to analyze NAc processing of the tone-cue during the Maintenance phase of SA in late sessions. Subjects that did not retain catheter patency or were otherwise unable to complete the entire course of SA were removed from the study. Additionally, sessions in which the animal was not "on task" were removed (defined as less than 5 Maintenance Hits, or more than 25 trials where drug level declined to 0 during Maintenance). In order to provide sufficient N for comparisons, all within session comparisons between Hits vs. Misses included only sessions with at least 5 Misses (excluding subjects with 100% Hits for example). The subjects finally included in the dataset (n=18) represented well-trained GT (n=7), NT (n=5), and ST (n=6) animals that completed the entirety of cocaine SA and contributed neural data.

3.3.1—Drug Level Regressed on a ST/GT pretest LP Continuum

It was of interest to identify if drug consumption differed along a basic continuum of LP. A linear regression was performed in R where the DV was specified as subjects' drug consumption (in mg/kg) during all late sessions and was regressed on individual STGT pretest LP results.

3.3.2— Escalation of Intake Over Sessions (ST, GT, NT)

Escalation of drug intake is known as a key measure of addiction in animal models of drug abuse, and it was important to identify if all groups escalated drug intake over time and if this rate of escalation differed between ST, GT and NT. The DV was subjects' drug consumption (in mg/kg) and was compared across **Early vs. Late sessions** and across **ST/NT/GT** using a repeated measures ANOVA in R.

3.3.3— Uncued Responding During Maintenance

Compulsive drug seeking (i.e., responding for drug when drug is unavailable) could be viewed as a risk factor for developing severe or uncontrollable drug addiction (Koob *et al*., 2004). Furthermore, this could be exaggerated among HI animals, due to the variable nature of drug availability in the present study. Using the calculation for URs (section 2.3.4) we generated the following four DVs (A-D) for each Late session and then compared these across ST, GT and NT groups, separately for Intake (HI and LI) using an ANOVA in R where IVs (STNTGT and Intake) were specified as categorical, and all possible two-way and three-way interactions were specified. The first analysis used the DV "**Total Time Responding in Corner**" and was defined as the total duration of all URs summed per session (A). The next analysis utilized the DV "**Total Uncued Responses**" and was defined as the total number of URs during the Maintenance phase of a session (B). We then calculated "**Rate of Uncued Responses Per Minute"** which was the total URs divided by the duration of the Maintenance session (in minutes) (C) Finally, **Average Uncued Response Duration** was defined as the average duration of all URs per

session (D). Due to interest in comparing all measures of UR between HI groups (GT vs. NT vs. ST), planned comparisons were performed for all calculated UR measures using the Tukey HSD test.

3.3.4— Maintenance Response Rate Analysis

We performed a subsequent two-part analysis of Response Rate comparisons for each categorical variable: Intake (HI and LI), Titration (Titrate and NoTitrate) and STNTGT (ST and NT and GT) using a generalized linear mixed model (GLMM) in SAS PROC GLIMMIX (SAS Institute, Cary NC). Each GLMM was run using robust standard errors and a gamma distribution with a log link was specified for the outcome.

The results of all omnibus GLMMs were reported but the major scientific purpose of the first analysis was to identify if Cued and Uncued Maintenance Response Rates differed for all possible combinations of independent variables (IV). Therefore, the first omnibus GLMM was run followed by planned post-hoc tests in which a test of the means between both Response Rates was performed for specific combinations of the different levels of the model's fixed effect categorical variables: e.g., GT HI NoTitrate Cued Response Rate vs. GT HI NoTitrate Uncued Response Rate. The purpose of this analysis was considered a first step in identifying tone-discrimination for each subcategory. The second analysis was performed in a similar manner but for just Uncued Maintenance Response Rate differences between Titrate and NoTitrate groups. Again, the results of the omnibus GLMM was reported for completeness, and planned post-hoc comparisons were made between means of Titrate and NoTitrate of the same

subcategory of Intake and STNTGT (e.g., Uncued Response Rate for GT HI NoTitrate vs. GT HI Titrate). The combination of results from both of these analyses would indicate how specific groups discriminated the tone.

3.3.5— Analysis of "Titration" During Maintenance

Based on literature on "titration" animals self-administer (Hit) when DL is lower than desired and avoid drug opportunities (Miss) when DL is above preferred DL as a way to control drug consumption. Analysis of titration was first computed for each session (DLDIFF, equation 5) where a positive DL difference value was expected and a negative DL difference value was unexpected and would be contrary to literature, as well as evidence of a possible inability to control drug intake. These sessions were carefully restricted to include only sessions with at least 5 Misses to avoid misinterpretations due to low N. Linear regressions were then performed in R where DLDIFF was specified as the DV and was regressed on average session DL separately for GT, NT, and ST during Late sessions.

3.4— Video Analysis during Cocaine SA

This study focused on NAc FR comparisons from the same neuron between Hits vs. Misses during the Maintenance phase. If the two FRs differed, it was important to identify if subject velocity (in centimeters travelled per second using diode tracking data) differed on Hits vs. Misses during the session, and also if DL impacted velocity.

3.4.1— Video Part 1: calculation procedures

Video Analysis Purpose: Potential problems with tone-evoked comparisons across Hits and Misses could be that NAc neurons are influenced by subjects' body movements (Coffey *et al.,* 2015). To address the influence of movement, we examined whether movement velocity was different on Hits vs. Misses averaged 1 second before the onset of the tone. We used high speed cameras to track a single brightly colored pixel on the animal's recording harness and converted this velocity data into centimeters travelled per second (CM/s) using the following procedure:

Video Recording: all video was recorded by a front-facing camera positioned outside the Plexiglas SA chamber. Each subject had a brightly colored diode affixed to their recording harness and this diode was used to track the rat's location 30 times per second for the entire 6 hour session using Datawave video recording software (Datawave Technologies, Longmont, CO) and positional data were input into the computer in x and y pixel coordinates. Only data from sessions were included if the following conditions were met: 1) Animal self-administered cocaine (see behavioral methods), 2) the camera angle must be straight (i.e., not rotated), 3) the video must include the entire operant chamber, and 4) the tracking diode on the recording harness must be trackable for the entire session.

Pixel Analysis: For each session, the video screen was divided into equivalent four quadrants (Fig. 4) where the top left quadrant (Q1) included the operant corner and consisted of 25% of the total video screen. Q2/3/4 were all other unpaired corners of the operant box. The distinct color of diode (bright orange/pink) was selected using the dropper tool to select a uniquely colored pixel to track over the entire course of SA.

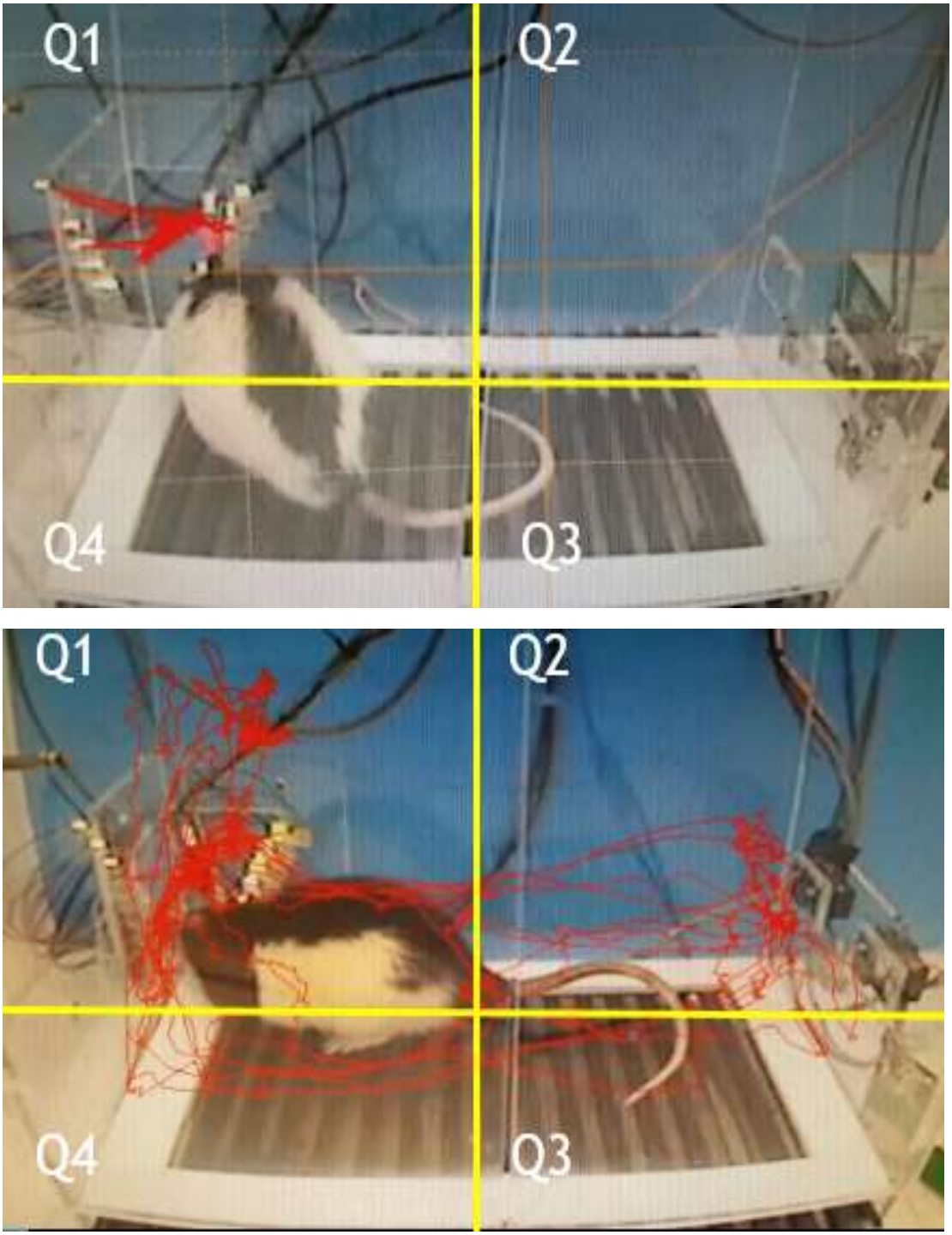

Figure 4: Video Analysis during cocaine SA: Video was recorded during all sessions in which neurons were recorded. A brightly colored diode was attached to the animal's headstage and was tracked every 33ms (the orange lines in the above figures). The screen was divided into four quadrants where Q1 was the operant corner to identify subject location (in or out of Q1) during SA.

Every time a unique pixel was identified as “tracking” the current location of the diode, the following data were output every 33ms: 1) the exact location of the animal in pixels (x and y coordinates) 2) the specific quadrant of the pixel location (Q1/2/3/4) and 3) the exact time (in milliseconds) that the diode was tracked.

Pixel to CM conversion: cameras output location data in a two dimensional format where an x and y value for the diode was tracked over the entire session every 33ms. Converting these data from pixel values to cm was performed in a multi-stage process to determine velocity (cm/second) of animal movements. 1) Separate X and Y values were calculated for the front quadrants (Q3 and Q4) and for the back quadrants (Q1 and Q2) using a recording harness that was disconnected from an animal. 2) The experimenter wore all black clothing and black nitrile gloves so the camera did not pick up any possible color interference. 3) Black dashes were made for CM markings in the front and back of the box for X and Y positions of the box and the experimenter slowly made passes along front and back x and y axis. Each pass was repeated 5 times for each video (totaling 20 passes) for 3 separate videos for a total of 60 passes. 4) Using the same parameters as a typical SA video analysis in Datawave, the total pixels travelled were converted to CM separately for each X and Y, front and back quadrants for each video 5) These values were then averaged over all videos for a final conversion factor for the front quadrants (Q3 and Q4, x=14.67 pixels/cm, y=13.59 pixels/cm) and the back quadrants (Q1 and Q2, x=10.41 pixels/cm, y=8.16 pixels/cm) to be used for subsequent analysis of velocity in cm/s.

Velocity Analysis: velocity of the animal's movement was calculated every second using the following equations separately for front (Q3, Q4) and back (Q1, Q2) quadrants:

$$Back\ Velocity\ \left(\frac{CM}{S}\right) = \left((x2 - x1) * \left(\frac{1}{10.4}\right)\right)^2 + \left((y2 - y1) * \left(\frac{1}{8.16}\right)\right)^2 \quad (7)$$

$$Front\ Velocity\ \left(\frac{CM}{S}\right) = \left((x2 - x1) * \left(\frac{1}{14.67}\right)\right)^2 + \left((y2 - y1) * \left(\frac{1}{13.59}\right)\right)^2 \quad (8)$$

In both instances x2 refers to the horizontal location of the animal (in pixels) 1 second prior to x1 location of the animal. Y2 and y1 were the same measures for vertical location of the animal. The conversion factors were from the pixel to cm calculations found for back and front quadrants. These horizontal and vertical vectors were then summed to calculate the overall distance traveled by the animal for 1 second of time to express these values in cm/s.

3.4.2— Video Part 2: analysis procedures

Velocity on Hits vs. Misses: The initial purpose of calculating velocity of the animal was to determine if velocity immediately prior to Hit trials was different from the velocity immediately before Miss trials during the same session. Using equations 6 and 7, velocity was first calculated for every second during the entire 6-hour SA session. Next, velocity was identified 1 second before the onset of the tone and this was then averaged for the entire Maintenance phase separately for Hit trials and Miss trials. For the analyses, we first performed a linear regression of velocity on Hits vs. velocity on Misses in R and then compared this slope vs. slope=1. Next, we ran a repeated measures

ANOVA in R where DV time 1 was equal to velocity on Hits and DV time 2 was equal to velocity on Misses and categorical IVs were STNTGT and Intake with posthoc analyses run on any significant main effects or interactions.

Velocity and DL: The other purpose of velocity comparisons was to determine if DL influenced velocity during the session. Studies have shown that administration of lower dose cocaine and other psychomotor stimulants increase forward whole-body movements, overall velocity, and rearing (Post & Rose, 1976) but this movement is replaced by quicker, focused stereotypic movements at higher drug levels (Dafny et. al., 1996). Identifying if movement was influenced by DL was important because accumbens FR can be correlated with DL. (Peoples et. al., 1998; Nicola & Deadwyler, 2000). Therefore, a linear regression was performed in R where average session Velocity was regressed on average session DL, separately for Hits and Misses.

% of Session in Operant Corner: The final video analysis was conducted on the % of time spent in the operant corner (Q1), and tested whether this time was influenced by the average DL maintained during maintenance of SA. We first calculated the total Maintenance time (in seconds) of the session that the animal stayed in Q1 and divided this by the total Maintenance time (in seconds) and multiplied this by 100. A regression analysis was performed in R where the DV was specified as the % of Session in Q1 and the IV was DL.

3.5— Neural Analyses: Data Preparation

3.5.0— Histological Identification of NAc Core and Shell

Following the final day of SA, localization of individual microwires (i.e., NAc core, shell, other) was performed. Animals were euthanized with a lethal dose of sodium pentobarbital (150-200 mg/kg i.p.) and a 4 sec, 50mA current was applied to each microwire hole (leaving trace amount of iron to be stained as a way to identify the exact location of all microwires). The animal was then perfused with saline and 4% paraformaldehyde where the brain was removed and stored in the same concentration of paraformaldehyde for two days. Whole brains were then transferred to a 30% sucrose-solution for several days and sectioned (50 µm) through the NAc with an extra ~1.5 mm rostral and caudal to ensure each wire was accounted for. Sliced tissue was then mounted and organized anterior to posterior and stained with calbindin d-28k which allowed for differentiation between NAc core and shell, and iron deposits were stained with 5% potassium ferrocyanide and 10% HCL. Each slice was then photographed and all 16 microwire tips were required to be accounted for. 'Missing wires' or wires located outside the NAc were discarded.

3.5.1— Extracellular Recording and Analysis

Single NAc neurons were recorded on alternating days in order to study individual NAc neuron processing related specifically to the onset of the cocaine availability cue (tone S^D). All neural signals were filtered through a customized preamplifier and stored offline for analysis using specialized SciWorks spike-sorting software (Datawave Technologies, Longmont, CO). Identification of signal from background noise was identified by waveform parameters: peak time, peak voltage, and spike height. Standard practice was to analyze FR around a repeated event and we used

the onset of the S^D tone-cue to extrapolate FR patterns. For each trial "pre-tone" firing rate (FR) (**Baseline**, -200ms) was compared to "tone-evoked" changes in FR (**Posttone**, +200ms) and FR was calculated as the number of discharges in each 200ms window. The 200ms firing window was chosen because it corresponded to the earliest onset of tone-evoked movements derived from extensive video analysis (see below, **3.5.2**) and was assumed to represent decisive cue processing during the critical early stages of "limbic-motor integration" (Nauta *et al.,* 1978) suggestive of neural activity flowing sequentially through laterally spiraling circuitry (Haber *et al.,* 2000). This approach was based on the assumption that neural processing of the tone 1) could be confounded by processing movement, and/or 2) might be finished once the animal started its movement toward the operant manipulandum.

3.5.2—Identification of a standardized 200ms "window"

Our experiment was designed to identify changes in NAc FR patterns evoked exclusively from the onset of the tone during the Maintenance period of SA, using tone onset as the node. We analyzed a significant portion of the dataset using two different procedures to determine if FR differed when we used 1) a fixed 200ms baseline coupled with a same-duration 200ms posttone firing window vs. 2) a firing baseline and posttone window that was customized for each trial, according to the onset of tone-evoked movement on that trial; frame-by-frame video-analysis determined the onset of tone-evoked movement, which defined the end of the posttone firing window on each trial, and also defined the duration of the preceding, baseline firing window for that trial. This was a critical comparison because each single trial required ~1 minute of frame by frame

analysis; analysis of all trials (n=59,562) would require almost 1000 hours of intensive labor. A paired-samples t-test conducted on the dataset (a subset of 19,333 trials) compared change scores ((PosttoneFR-BaselineFR)/(PosttoneFR-BaselineFR+.1)) between procedures 1 and 2 above. There was no significant difference in FR for videoscored data (M=.00018, SD=.124) vs a fixed 200ms firing window (M=.0304, SD=.487) procedures; $t(573)=1.75$, $p > 0.05$. Therefore, because there was no difference, FR was assessed utilizing a standardized 200ms for all subsequent analysis of NAc neurons (Fig. 5).

3.5.3—Tracking the same neuron over sessions

Recording the same neuron over sessions was vital to this study. The NAc's principal neurons, which comprise 95% of its neuronal population, are slow firing, medium spiny neurons (MSNs) (Kemp, Powell 1971). Interpretations of similarity are informed by the fact that MSNs are readily identified by waveform (Kulik *et al,* 2017). Our lab has demonstrated stability of single unit recording over sessions (Peoples *et al.,* 1999; Tang *et al.,* 2008; Coffey *et al.,* 2015) and this study utilized the same parameters: 1) Waveform must be recorded from the same microwire; 2) Waveform and ISI histogram must be similar across days and the correlation between average waveform voltages during the spike must be >0.9; 3) The parameters of the waveform must be similar, i.e., the differences in spike height between sessions must be <20%, the difference in peak time from one session to the next <.04ms, and the waveform not different based on visual inspection by a trained observer; 4) Neural discharge must not occur within the first 2 msec of the ISI histogram, i.e., evidence of a single neuron's natural refractory

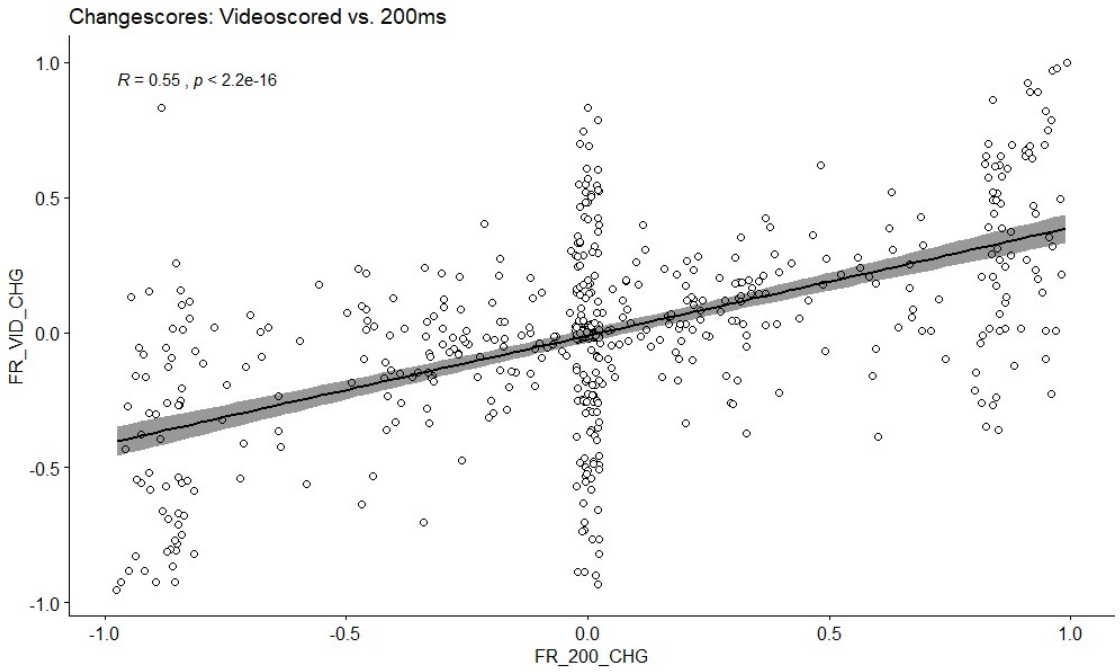

Figure 5: Does a neuron's tone-evoked change in FR using standardized 200ms baseline and posttone windows, differ from its tone-evoked change using "video-scored" baseline and posttone windows (time of tone onset until the first tone-evoked movement)? Each dot represents a single neuron's tone-evoked FR change scores ((PosttoneFR-BaselineFR)/(PosttoneFR-BaselineFR+.1)). The y axis is an assessment utilizing videoscored FR, regressed on data using a fixed 200ms window (x axis). There was no significant difference between videoscored FR and FR assessed on a standardized 200ms window.

period (Kosobud, Harris & Chapin, 1994); 5) In the uncommon event of two different units on the same wire: If the second unit met all criteria for a single neuron, and fired within 2msec after the first neuron's discharge, then it represented a second individual neuron. Any recordings which failed to meet any of these criteria were discarded. Loss of stability was also readily recognized using these criteria, after which data recorded from that wire were discarded. The effectiveness of applying these criteria, developed in this laboratory over the past 20 years, is illustrated in Figure 6 and a **Neuron** was therefore defined as all Maintenance trials for all late sessions in which the same neuron was recorded.

3.6— Neural Analyses: Data Analysis

All neural analyses were restricted to Late sessions in which the animal was "on-task" (see behavioral analyses), and any comparisons across Response required a minimum of 4 Misses during the Maintenance phase of SA.

All collected neural data had a hierarchical structure in which FRs from individual trials were "nested" within the same neuron (Raudenbush & Bryk, 2002; Bolker *et al.*, 2009; Stroup, 2012). Traditional ANOVA models would not be suitable for the nested data due to violation of the assumption of independence and would otherwise lead to an inflated Type I error rate (Raudenbush & Bryk, 2002; Bolker *et al.*, 2009; Stroup, 2012). Therefore, linear mixed models (LMM) were run in which all individual Posttone trial FRs were included and Neuron was specified as a random effect. All omnibus

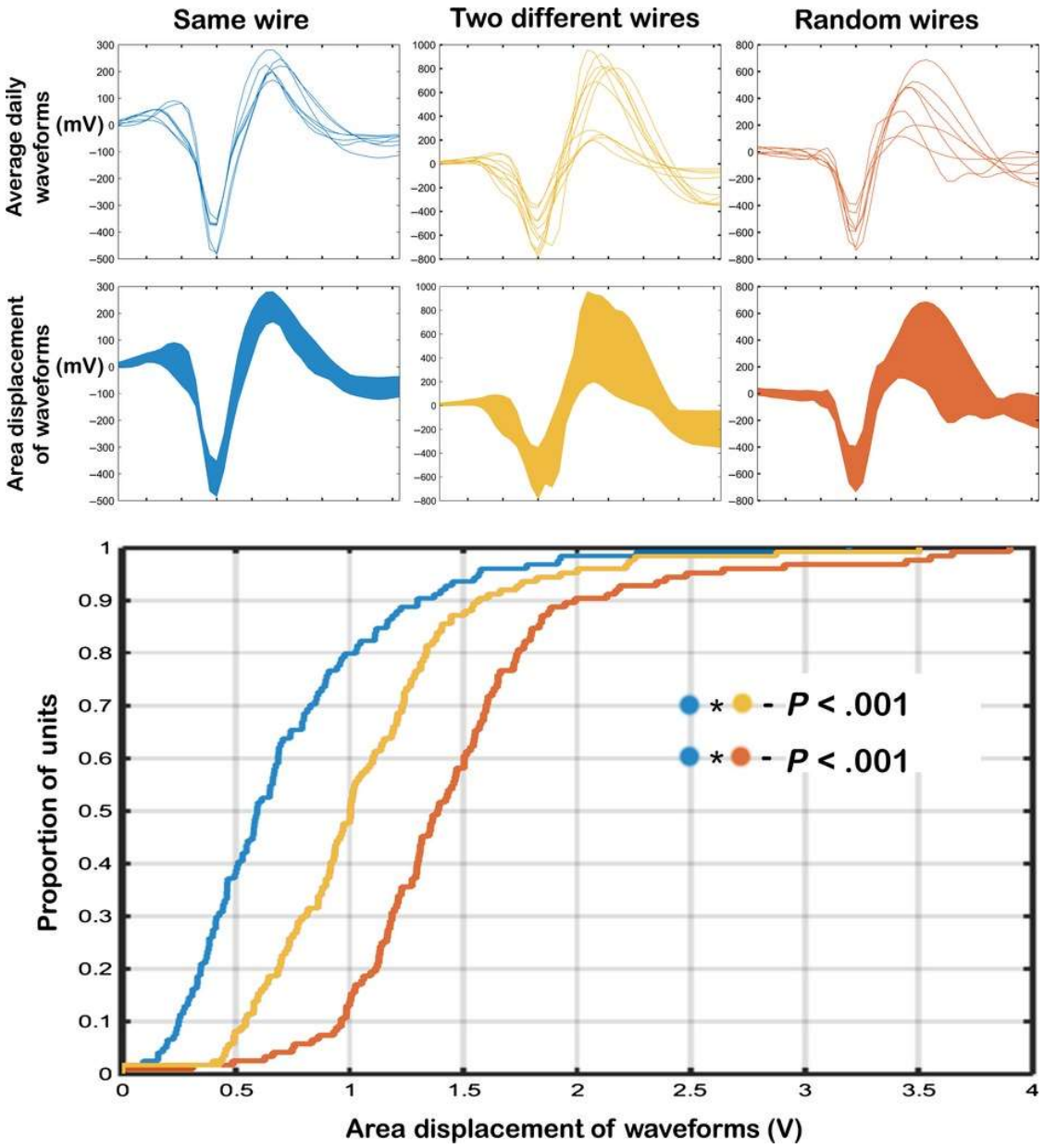

Figure 6: Visual representation of neurons considered to be the same neuron across trials according to accepted statistical criteria in the field (criteria and figure from Coffey *et al.*, 2015). Neuron waveforms recorded from the same wire across days overlap and display a lower "noise band", resulting in less "area displacement of waveforms (V)" (blue) while neuron waveforms recorded from different wires produce significantly different electrophysiological characteristics (yellow and orange), and are readily identified as "different" neurons with a high degree of V.

analyses were run using SAS PROC MIXED and all post-hocs analyses were run using PROC PLM (SAS Institute, Cary NC).

For each LMM all possible combinations of independent variables (IV) were specified and all analyses utilized robust standard errors. Each LMM included **Average Baseline FR** as a continuous IV that was calculated for each Neuron and categorical variables such as **Response** (Hit and Miss) and **Intake** (HI and LI). The specific models are delineated below. For each model, robust standard errors and an unstructured covariance matrix (type=UN) for the random effects were specified. The goal for each of these analyses was to model or analyze the "stability" of FR across **Tone** (Baseline vs. Posttone FR), i.e., how much correlation (or "stability") existed between Time2 (Posttone) and Time1 (Baseline) FRs, as a regression slope for each specific categorical subgrouping in the research design.

For the initial stability analysis 95% confidence intervals were computed for regression slopes and intercepts. All confidence intervals were adjusted using the Holm-Sidak post-hoc correction for multiple tests in order to control for Type I error. The confidence intervals were used to test whether the slopes were different from 1.0 and 0. Stability was defined by a slope of 1.0 (i.e., no consistent change from Baseline to Posttone). If a slope showed statistical difference from 1.0 then the relationship was considered to lack stability, and for the purpose of this study was considered "**tone-evoked**". If the slope showed no statistical difference from 0 then there was no relationship whatsoever between Baseline and Posttone FR and was also considered

"**tone-evoked.**" Anything resulting in a "not-stable" finding was considered sufficient evidence of tone-evoked changes (i.e., the entire purpose of this study).

While the results of the omnibus LMMs are reported, they were not of theoretical scientific interest because (as outlined above in the introduction) our main substantive interest was to analyze "stability" of FRs across Tone (Baseline vs. Posttone) for each specific subcategory (e.g., GT HI core Hit) and then determine if this "stability" differed across Response for the same subcategory of interest, e.g., GT HI core Hit slope vs. GT HI core Miss slope. As such, planned post-hoc comparisons between Hits vs. Misses were specified a priori regardless of whether omnibus interactions and main effects showed significance in the initial LMM. Furthermore, post-hoc comparisons of Response were compared only for the specific subcategory (e.g., core, HI, GT) if either Hit or Miss (or both) were "tone-evoked" to identify if the tone was differentially processed across different Responses (Hits vs. Misses).

There were *three* distinct phases of stability analysis:

Phase 1: Does the onset of the tone evoke a change in FR (Baseline vs. Posttone)?

For the first phase, Average Baseline FR was computed for each unique Neuron across all late Maintenance trials (across all sessions in which it was recorded, from sessions 4-15) separately for each fixed effect categorical variable: Region (core and shell), Intake (HI and LI), and STNTGT (ST and NT and GT). The omnibus LMM was followed by post-hoc tests in which the linear regression stability model (Posttone FR regressed onto Average Baseline FR) was run for each possible combination of the

different levels of the model's fixed effect categorical variables. The results of the Phase 1 stability analysis were reported but no post-hoc analyses were specified as Response was purposely collapsed. The purpose of this analysis was to identify if the tone was processed whatsoever, ignoring Response entirely.

Phase 2: Does stability vary across Response?

The second phase was performed in a similar manner as phase 1 where Average Baseline FR was computed for each unique Neuron across all late Maintenance trials separately for each fixed effect categorical variable: Region, Intake, STNTGT, except Phase 2 now included Response (Hits and Misses) as an additional categorical variable. The omnibus LMM was followed by post-hoc tests in which the linear regression stability model (Posttone regressed onto Average Baseline FR) was performed for each possible combination of the different levels of the model's fixed effect categorical variables. The results of the Phase 2 stability analysis were reported, and planned post-hoc comparisons were made of the regression slopes between Hits and Misses for each subcategory (Region, Intake, STNTGT). The purpose of this analysis was to identify if tone-processing was evident only on Hits or Misses, and to then compare if tone-processing was different across Hits vs. Misses.

Phase 2 also included an analysis of Trial level Baseline FR comparisons made between only Hits and Misses for each unique aforementioned subcategory of the IVs. Because the outcome variable (trial Baseline FR) showed skewness, a constant value of 1 was added and a generalized linear mixed model (GLMM) was run in which a gamma

distribution with a log link was specified for the outcome. When using the GLIMM model we were able to preserve the "Zero" aspect of the raw data. The DV (in this case, trial level Baseline FR) was "mapped" onto a log scale when running the GLMM (i.e., specifying a log link with gamma distribution). A log of 0.1 results in a "negative" value (-1) and a log of 1.0 is "zero." In this way, we were able to preserve the "zero" value of the raw data because the "log-transform" that takes place under the hood in the GLMM via the log-link could not handle a "zero" value (log of zero is undefined). In other words, the raw data value of "zero" was kept "zero" by adding a 1.0, but if we added a 0.1 then that value would become "-1." The omnibus GLMM was run followed by planned post-hoc tests in which a test of the means between Hits vs. Misses was performed for each possible combination of the different levels of the model's fixed effect categorical variables. All analyses were performed using SAS PROC GLIMMIX. Results from this analysis was essentially a control to identify if elevated (or suppressed) Baseline FR differences on Hits vs. Misses contributed to overall tone-evoked differences in Hits vs. Misses.

Phase 3: Does "Titration" influence stability across Response?

The third phase was performed in a similar manner as phase 2 where Average Baseline FR was computed for each unique Neuron across all late Maintenance trials separately for each fixed effect categorical variable: Region, Intake, STNTGT, Response, except Phase 3 now included Titration (Titrate and NoTitrate) as an additional categorical variable. The Titration variable was specified for each session, and was a method of identifying the effect of DL on Hit vs. Miss Tone processing (i.e., the category

Titrate was associated with a high Miss DL, while the category NoTitrate was associated with a high Hit DL for that session). The omnibus LMM was run followed by posthoc tests in which the linear regression stability model (Posttone regressed onto Average Baseline FR) was performed for each possible combination of the different levels of the model's fixed effect categorical variables. The results of the Phase 3 stability analysis were reported and planned post hoc comparisons were made of the regression slopes between Hits and Misses for each subcategory (Region, Intake, STNTGT, Titration).

Phase 3 also included an analysis of Trial level Baseline FR comparisons made between only Hits and Misses for each unique aforementioned subcategory of the IVs. Because the outcome variable (trial Baseline FR) showed skewness, a constant value of 1 was added to preserve the "zero" nature of the data (see the explanation in Phase 2 baseline analysis) and a generalized linear mixed model (GLMM) was run in which a gamma distribution with a log link was specified for the outcome. The omnibus GLMM was run followed by planned post-hoc tests in which a test of the means between Hits vs. Misses was performed for each possible combination of the different levels of the model's fixed effect categorical variables. All analyses were performed using SAS PROC GLIMMIX.

4.0 –Results

4.1-- STGT Phenotype identification

4.1—LP as a definitive measure of Goal, Non, or Sign tracking: Composite Score

To confirm that low LP animals were actually Goal-Tracking, Composite Score was regressed on LP during the final day of the STGT pretest and the regression was significant ($F(1,18)=5.35$, $p=.03$), R^2 of .23, slope=-.01 and intercept=6.91 (Fig. 7). Therefore, a low number of LP was considered an appropriate measure of Goal-tracking for subsequent analyses.

4.2—Self-Administration: Behavioral results

4.2.1— Acquisition of Predrug Tone-Discrimination

The purpose of this study was to identify NAc processing of the tone-cue S^D, and it was important to determine if animals acquired tone discrimination across **Sessions**, and if this differed between GT, NT and ST. The repeated measures ANOVA yielded a significant main effect of **Response-Rate** (Cued vs. Uncued) $F(1,324)=6.67$, $p=.01$ but no main effect of **STNTGT** $F(1,324)=1.24$, $p>.10$**, Sessions** $F(1,324)=2.33$, $p>.10$**,** or interactions. These results suggest that ST, GT, and NT increase Cued Predrug Response Rate with no change in Uncued Predrug Response Rate over sessions, suggesting equivalent group acquisition of tone discrimination as a function of training (Fig. 8).

4.2.2—Drug Consumption Regressed on LP achieved during the pretest

It was important to identify if drug consumption changed as a function of pretest LP; i.e., how does the degree of “Sign-Tracking” impact the total cocaine consumed over the entire course of SA? The regression was not significant ($F(1,73)=1.14$, $p>.10$, slope=-1.0 and intercept=139.6) with $R^2 = .015$, indicating no correlation between drug

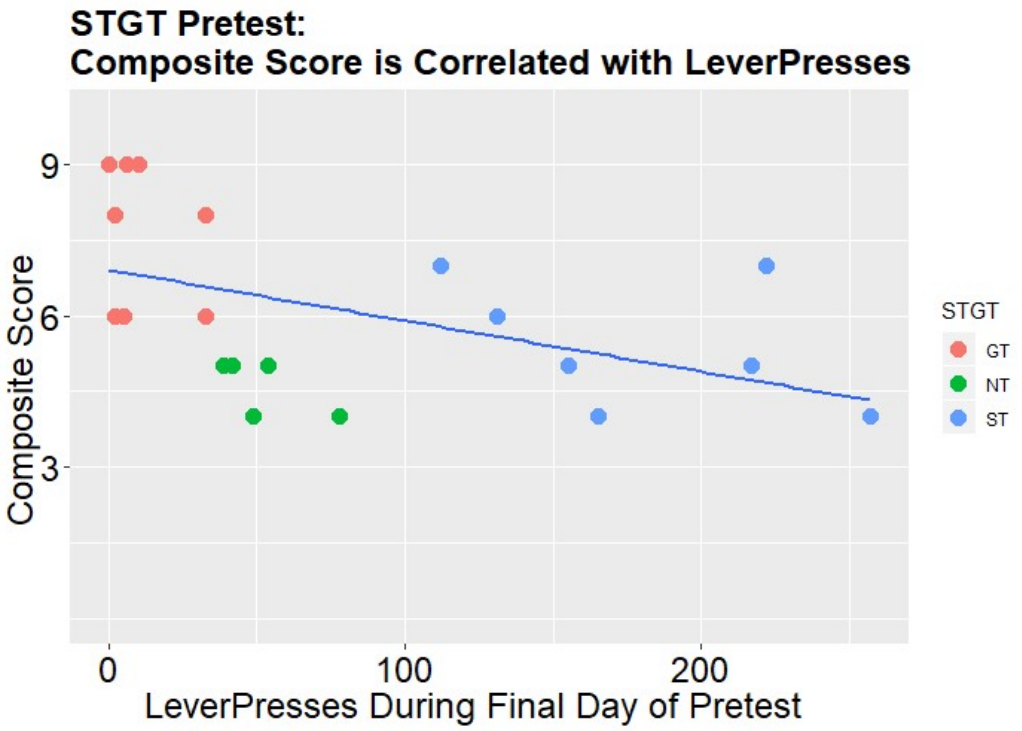

Figure 7: Confirmation of LeverPressing as a definitive measure of Goal, Non, or Sign tracking: The composite score was created by combining "rank" score of three distinct measures of Goal-Tracking during the final session of the STGT pretest. These ranked measures included total FoodPort entries, average latency to enter the foodport, and total LeverPresses. Each dot represents one subject and the y axis is the sum of the three scores and a total score of 9 indicated strong Goal-Tracking tendencies and a low score as strong Sign-Tracking tendencies. Composite score was regressed on LeverPresses during the final day of the STGT pretest (x axis) and was significant ($f(1,18)=5.35$, $p=.03$), with an R^2 of .23, slope=-.01 and intercept=6.91. Therefore, a low number of LeverPresses could be considered an appropriate measure of Goal-tracking.

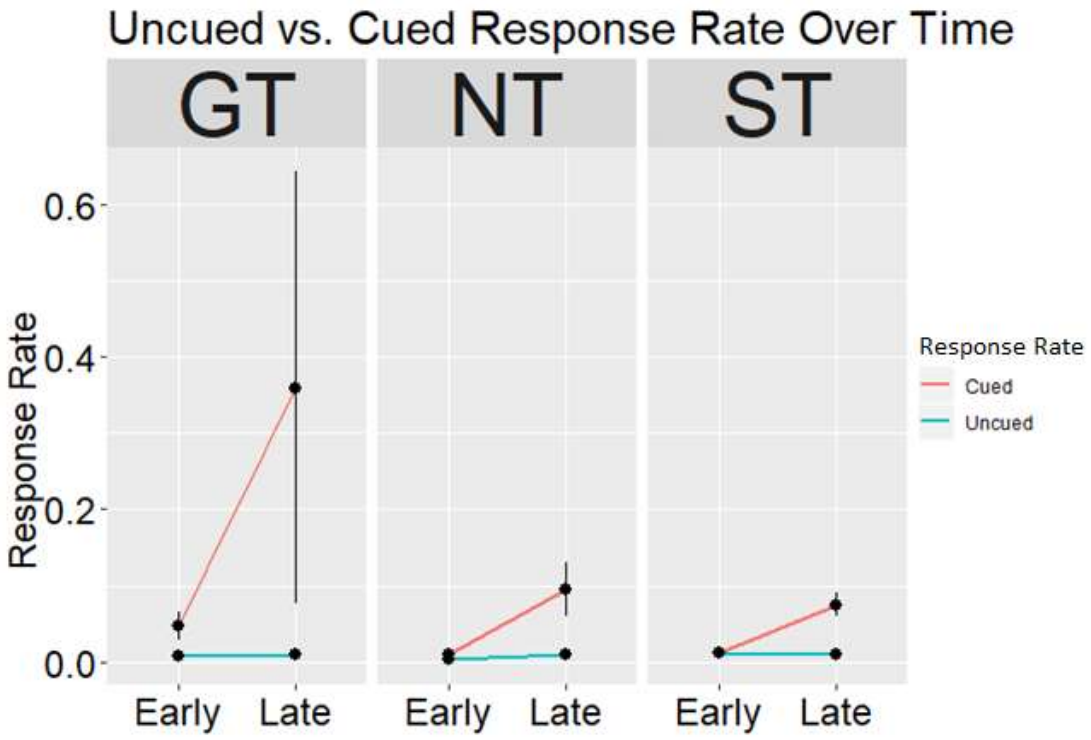

Figure 8: Acquisition of selective tone discrimination before the first daily infusion. Response rate was first calculated separately for cued and uncued response rate individually for each subject and session and compared early vs. late and across STNTGT. All groups increased in Cued response rate over sessions (red line). All groups also did not increase uncued response rate over sessions (blue line). Therefore, all groups acquired selective tone-discrimination.

consumption and LP (Fig. 9). Therefore, drug consumption was not predicted by Sign or Goal phenotype measured by pretest LP.

4.2.3— Escalation of Intake Over Time (ST, GT, NT)

Escalation of drug consumption was an identifiable measure in the present study and is known as an important identifier of "addiction" in animal models. The repeated measures ANOVA revealed GT, ST, and NT groups increased drug consumption between **Early vs. Late** sessions $F(1, 26)=12.78$, $p=.001$, which suggested cocaine escalation, but the rate of escalation did not differ between groups; no significant main effect of **STNTGT**, $F(2, 34) = 1.03$, $p. > .10$, nor a significant interaction of **Session** by **STNTGT**, $F(2, 34) = 2.34$, $p > .10$ was observed. Therefore, all groups acquired cocaine self-administration and escalated drug intake at similar rates (Fig. 10).

High Intake Analysis: It should also be noted that ST had a lower rate of HI sessions revealed by an ANOVA in R. There was a significant main effect of STGT $F(2, 190)=5.66$, $p=.004$ and post hoc tests revealed differences between ST and GT ($p=.003$) but not GT and NT ($p>.10$). Although this did not impact other aspects of drug intake across groups, it is one of several findings herein indicating that the GT phenotype must be considered at risk for cocaine abuse.

4.2.4— Analysis of Titration During Maintenance

Inability to "titrate" or control drug intake is a major risk factor for development of addiction (Koob *et al.,* 2004) and differences in expected SA patterns could point to

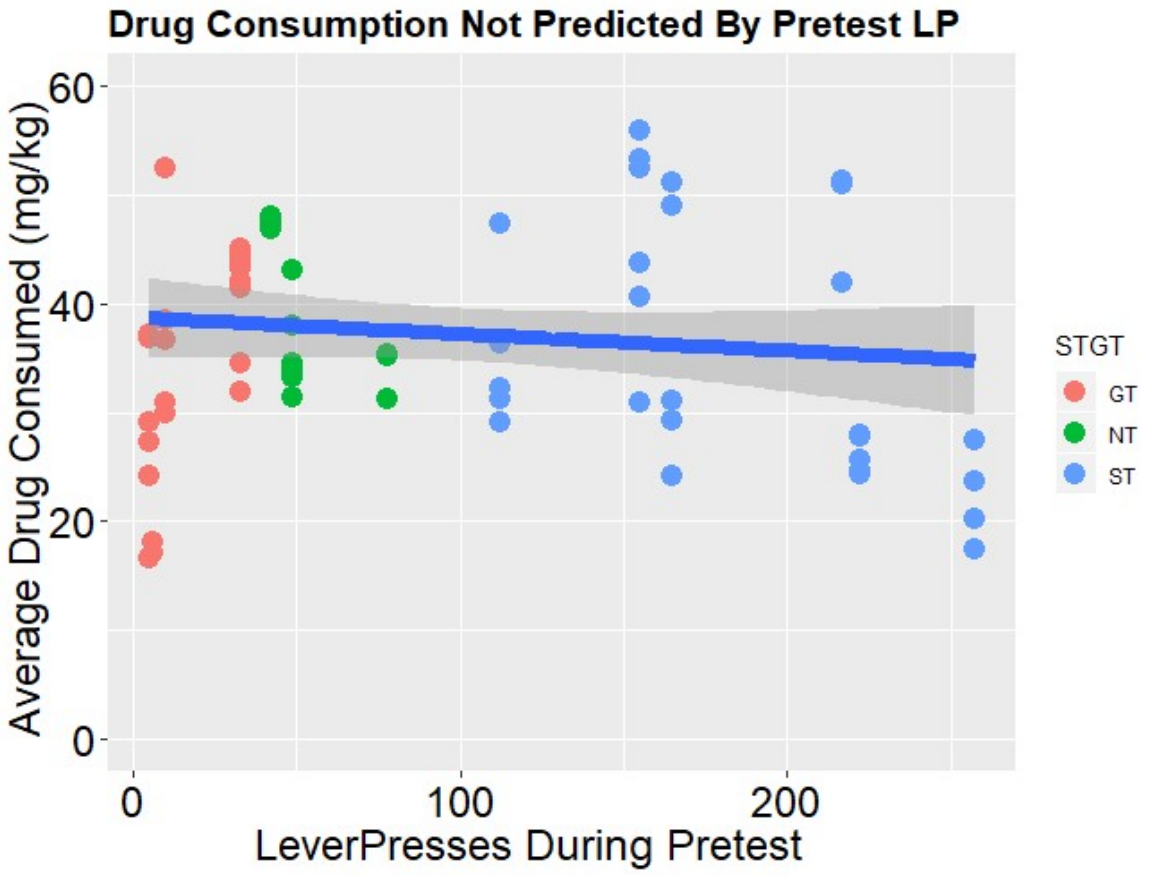

Figure 9: LeverPresses during the STGT pretest do not predict average drug consumption during late sessions. Each dot represents one animal and the color indicates ST, GT, or NT phenotype. The y-axis represents the average drug consumption during the maintenance phase of all late sessions of cocaine SA, and the x-axis is Subject LeverPresses identified on the last day of the STGT pretest. A linear regression was conducted to predict average DL based on LeverPresses. The regression was not significant ($F(1,73)=1.14$, $p>.10$) with $R^2 = .015$. The slope was calculated as -1.0 and intercept=139.6. Therefore, drug consumption is not predicted by Sign or Goal-tracking phenotype measured by pretest LeverPresses.

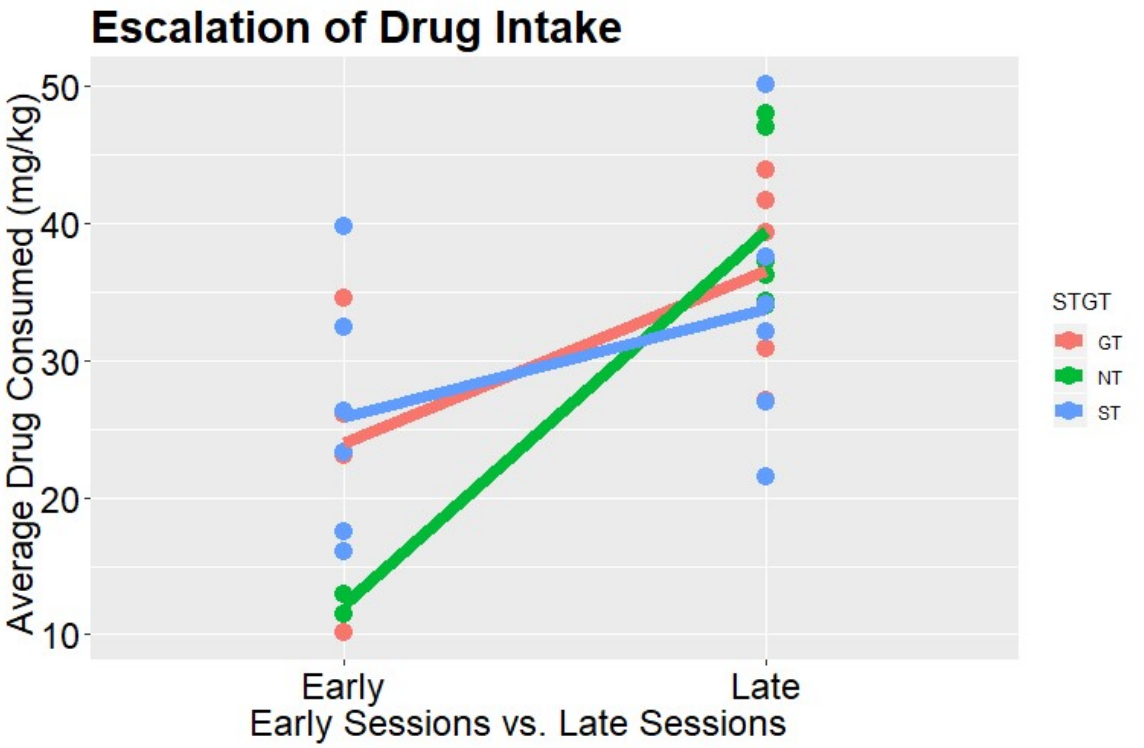

Figure 10: Escalation of Cocaine Intake over time. Each dot represents subject's average drug consumed (mg/kg) for early sessions (1-3) or late sessions (>4) across the entirety of cocaine SA. GT, ST, and NT groups escalated drug intake over Sessions (Early vs. Late) but the rate of escalation did not differ between ST, GT, and NT groups. Therefore, all groups acquired cocaine self-administration and escalated cocaine intake at similar rates.

increased risk of developing severe substance abuse. Analysis of titration was computed for each session (DLDIFF, equation 5) where a positive DL difference value (**Titrate**) was expected and a negative DL difference value (**NoTitrate**) was unexpected and would be contrary to titration literature. The distribution of DLDIFF as a function of average session DL among GT, NT, and ST was performed using a linear regression in R where DLDIFF was specified as the DV and regressed on average session DL separately for GT, NT, and ST during Late sessions (Fig. 11). For GT, the regression was significant ($F(1,66)=24.88$, $p<.001$) with $R^2 = .27$. The slope was calculated as -.422 and intercept=3.69 suggesting an ability to titrate at low drug levels but not High drug levels. For ST, the regression was significant ($F(1,67)=20.17$, $p<.001$) with $R^2 = .23$. The slope was calculated as -.423 and intercept=3.30 suggesting an ability to titrate at low drug levels but not high drug levels. For NT, the regression was not significant ($F(1,48)=.32$, $p>.10$) with $R^2 = .01$. The slope was calculated as .05 and intercept=-.05 suggesting NT were able to titrate across all intake levels, although the alternative explanation is that NT were equally poor at titrating across all drug levels (with negative DLDIFF scattered throughout the continuum). Results suggest both GT HI and ST HI (but not LI) were unable to control DL during the session.

4.2.5— Uncued Responding During Maintenance

Results from figure 8 suggested animals acquired the selective properties of the tone and task during the predrug phase of SA. However, during Maintenance, the randomized ITI ensured that animals were unable to predict the next tone onset time, and therefore unsure of when drug would suddenly become available to self-administer.

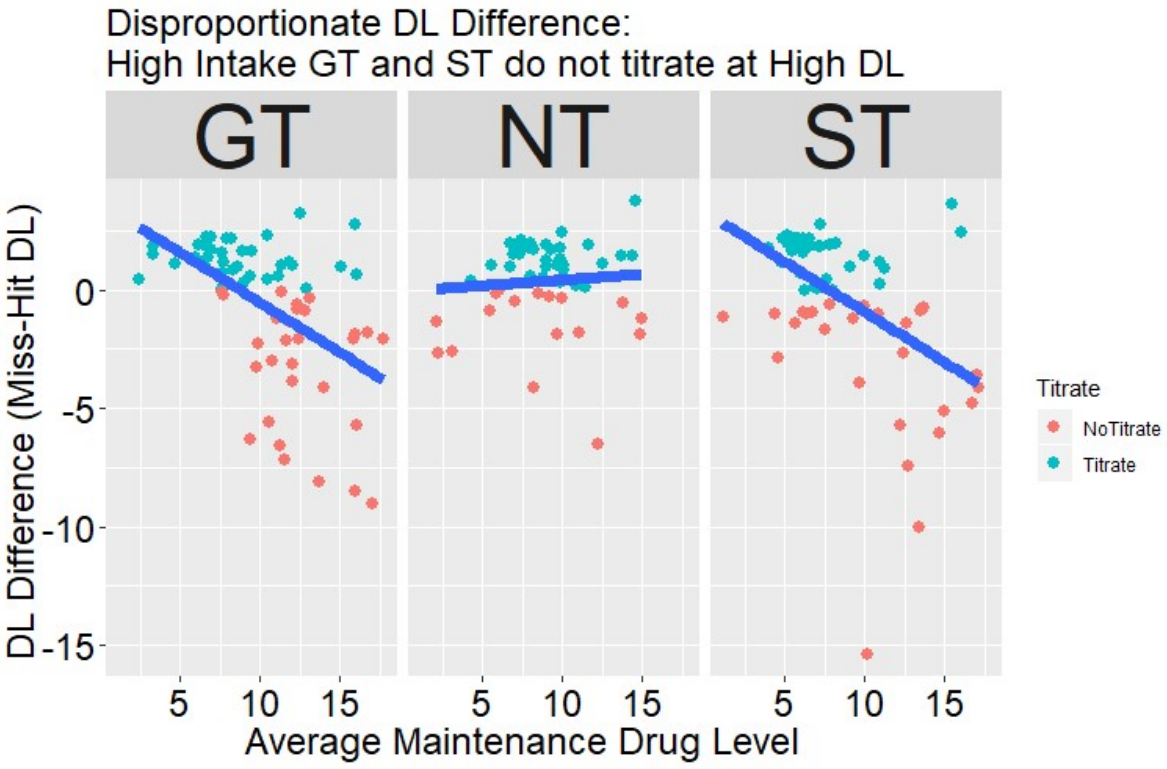

Figure 11: Analysis of "titration" as a function of Increasing DL: Analysis of "titration" was calculated for each session by subtracting the average drug level during Hit trials from Miss trials during the maintenance phase to create a drug level difference score (DLDIFF) indicated by the dot placement on the Y axis. Literature indicates animals take drugs to seek a "preferred" drug level within a given session where 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 DL difference value was expected, and considered sufficient evidence of "titration". A negative DL difference value was unexpected and would be contrary to literature, indicating a low Miss DL and high Hit DL in the same session. Results are unexpected to titration logic, and indicate that as a function of increasing drug intake both GT and ST had greater instances of "not-titrating" while NT demonstrated a consistent titration pattern across the entire spectrum of DL.

This modeled situations of unpredictability of drug availability in humans suffering from substance abuse disorder. Therefore, response rates when the tone was off (Uncued Responses, UR) provided a potential measure of drug craving during periods of drug unavailability. We were specifically interested in comparing the distribution of URs between HI groups. We identified the following (Fig. 12a-d): A) **Total Time Responding in Corner**: An ANOVA revealed significant main effects for STNTGT $F(2,186)=5.05$, $p=.007$) and for Intake (High Intake vs. Low Intake) $F(1,186)=41.84$, $p<.001$ but not a significant interaction effect of STNTGT and Intake $F(2,186=2.12$, $p>.10$. Due to a priori interest in comparisons between HI animals, post hoc analysis was conducted of the interaction between STNTGT and Intake. Significant differences were observed among GT HI vs. NT HI ($p=.019$) but not in ST HI vs. NT HI ($p>.10$) and not in GT HI vs. ST HI ($p>.10$) nor any LI group comparisons. B) **Total Uncued Responses**: An ANOVA revealed significant main effects for STNTGT ($F(2,186)=10.14$, $p<.001$) and for Intake ($F(1,186)=94.78$, $p<.001$) but not a significant interaction effect of STNTGT and Intake ($F(2,186)=1.42$, $p>.10$). Due to a priori interest in comparison of HI, post hoc analysis of the interaction of STNTGT and Intake did not reveal significant differences (GT HI, NT HI, ST HI were all not different). **C) Uncued Response Rate**: An ANOVA revealed significant main effects for STNTGT ($F(2,186)=8.88$, $p<.001$) and for Intake ($F(1,186)=90.02$, $p<.001$) but no significant interaction effect of STNTGT and Intake ($F(2,186)=1.64$, $p>.10$). Due to a priori interest in HI comparisons, post hoc analysis of the interaction of STNTGT and Intake (although close) did not reveal significant differences among GT HI vs. NT HI ($p=.078$), nor in ST HI vs. NT HI ($p>.10$), nor in GT HI vs. ST HI ($p>.10$), nor in LI group

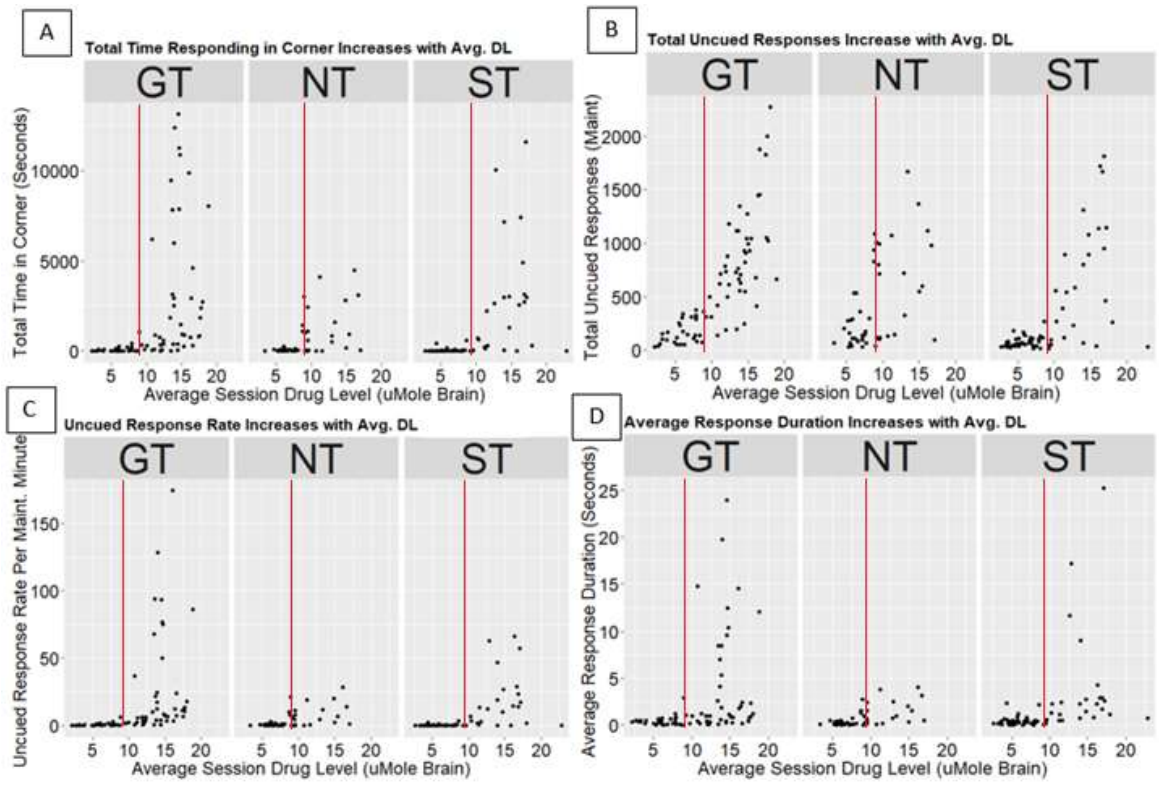

Figure 12: Uncued Response measures during late sessions increase with average DL. For each figure (12A-D) all UR measures (Y axis) significantly increased between LI and HI sessions (noted by the red intercept on the X axis) for all groups (ST, NT, GT). Furthermore, all LI groups were not different from one another (ST, NT, GT). Differences between HI groups only existed between GT HI and NT HI (12A, D) but not for GT HI vs. ST HI. This suggested subjects from HI groups continuously responded in the operant corner while the tone was off (and drug was unavailable).

comparisons. **D) Average Response Duration**: An ANOVA revealed significant main effects for STNTGT ($F(2,186)=3.82$, $p=.024$) and for Intake ($F(1,186)=25.39$, $p<.001$) but no significant interaction effect of STNTGT and Intake ($F(2,186)=1.77$, $p>.10$). Due to a priori interest in HI sessions, post hoc analysis of the interaction of STNTGT and Intake revealed significant differences among GT HI vs. NT HI ($p=.043$) but not between ST HI vs. NT HI and($p>.10$), nor in GT HI vs. ST HI ($p>.10$), nor in LI group comparisons. In all cases, HI vs. LI was different and all LI groups (ST vs. GT vs. NT) were not different from one another. Differences that emerged among HI groups were solely contributed by GT HI vs. NT HI (Fig. 12a, d) and no differences were apparent between GT HI and ST HI, or ST HI vs. NT HI. Therefore, GT HI (and ST HI) groups sought drug for long periods of time when the tone was off (and drug was unavailable). In general, HI subjects seemed to abandon Predrug tone-discrimination in favor of constantly responding in the operant corner.

Uncued Nosepokes Centered on Hit as the Node: This phenomenon of non-stop nosepoking is represented in Figure 13, which demonstrates that HI subjects (in particular HI NoTitrate subjects) performed a consistent pattern of short latency uncued nosepokes immediately prior to the tone onset on Hits. Examination of the raw data (Fig. 13) suggested a high degree of short latency Hits immediately following the onset of the tone for the same groups. This provided further evidence for lack of tone-discrimination among NoTitrate groups.

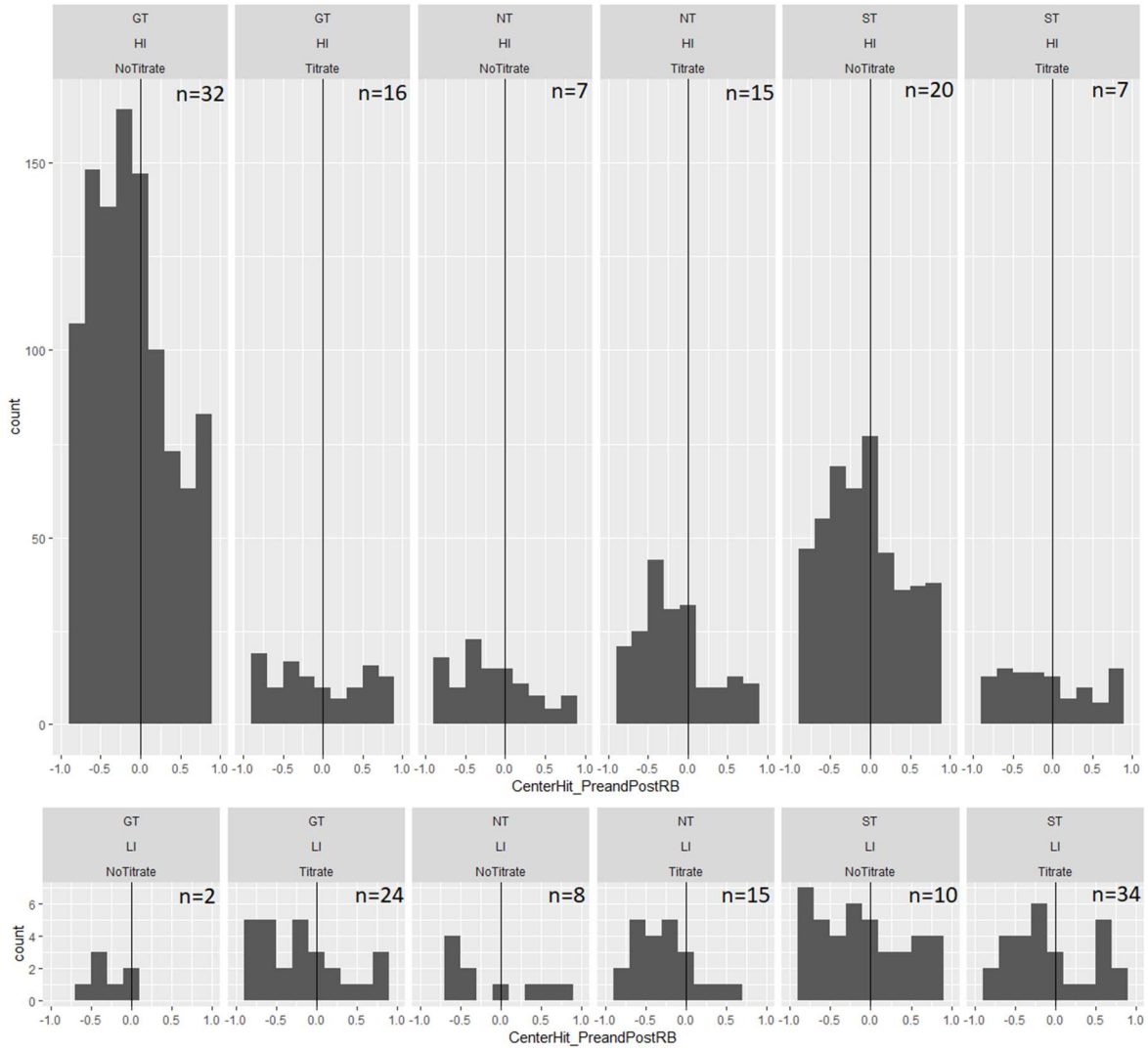

Figure 13: For HI, NoTitrate sessions both GT and ST demonstrated large numbers of short interval uncued nosepokes immediately before the onset of the tone on a Hit Response. The same groups demonstrate a drop in short latency intervals following the onset of the tone, which could be explained by the lack of a Hit response. Examination of the raw data confirmed as much, and adding a number of "short latency Hit responses" (i.e., <1 second) would flatten the dropoff following the onset of the tone. ST NoTitrate (55% of Hits) and GT NoTitrate (51% of Hits) were <1sec latency. These results suggest that the aforementioned groups may continuously nosepoke until the tone comes on and decrease in the rate of nosepoking upon the onset of the tone (and achieving the desired Hit).

4.2.6— Maintenance Response Rate Analysis

We then analyzed Cue Category (Cued vs. Uncued Maintenance Response Rate) differences for every category of IV (STNTGT, Intake, and Titration). For purposes of completeness the omnibus GLMM revealed significant Main effects for Intake $F(1,193)=38.07$, $p<.0001$ and Cue Category $F(1,193)=69.10$, $p<.0001$ but not STNTGT $F(2,193)=.44$, $p>.10$ or Titration $F(1,193)=1.68$, $p>.10$. Significant two way interactions were found for STNTGT by Titration $F(2,193)=3.20$, $p=.043$ and marginal significant results for STNTGT by Intake $F(2,193)=2.95$, $p=.055$ but not for Intake by Titration $F(1,193)=.36$, $p>.10$ or STNTGT by Cue Category $F(2,193)=.66$, $p>.10$ or Intake by Cue Category $F(1,193)=1.27$, $p>.10$ or Titration by Cue Category $F(1,193)=.51$, $p>.10$. There was a significant three way interaction for STNTGT by Intake by Titration $F(2,193)=4.99$, $p=.023$ but not for STNTGT by Intake by Cue Category $F(2, 193)=.61$, $p>.10$ or Intake by Titration by Cue Category $F(1, 193)=.73$, $p>.10$. The four way interaction was also not significant STNTGT by Intake by Titration by Cue Category $F(2, 193)=.38$, $p>.10$. Post hoc analyses were performed on the aforementioned group differences in Cue Category (Uncued vs. Cued Maintenance Response Rate) for each specific subcategory and revealed almost significant differences: GT HI NoTitrate ($p=.03$), GT HI Titrate ($p=.04$), GT LI Titrate ($p<.0001$), NT HI Titrate ($p=.0002$), NT LI NoTitrate ($p<.0001$), NT LI Titrate ($p<.0001$), ST HI NoTitrate ($p=.0001$) and ST HI Titrate ($p<.0001$), ST LI NoTitrate ($p=.028$) and ST LI Titrate ($p<.0001$). NT HI NoTitrate was the only non-significant result ($p>.10$). Results suggest a higher degree of Cued Response rate for almost all groups except for NT HI NoTitrate (Fig. 14), which suggests NT HI NoTitrate did not discriminate the tone.

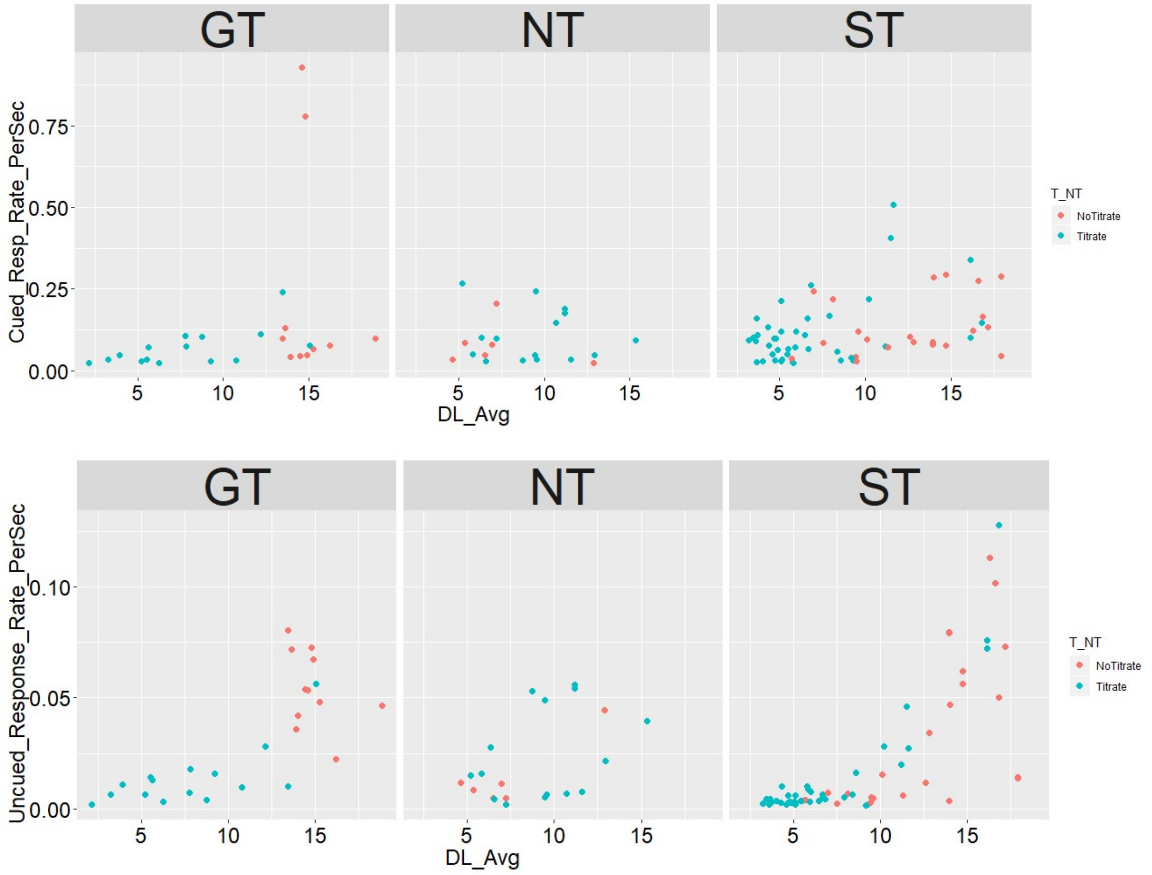

Figure 14: Maintenance Response Rate Analysis. The color of each dot represents the designation of a session as “Titrate” or “NoTitrate” and placement on the x axis is average drug level maintained by the animal. The Y axis in the upper figure is “Cued Response Rate” and the lower is “Uncued Response Rate” (see equations 5 and 6) attained during the Maintenance phase. Results suggested no difference in Cued vs. Uncued response rate for NT HI NoTitrate, and significantly higher Uncued response rate among GT HI NoTitrate relative to GT HI Titrate, but a consistently high (but no difference between Titration groups) rate of Uncued responding among HI ST.

However, the differences identified in Cued vs. Uncued Responding for the GT and ST HI NoTitrate groups were overshadowed by evidence of “constant nosepoking” identified Figure 13.

The second phase of the analysis was a direct comparison of Uncued Response rate for a specific category of STNTGT and Intake between Titrate and NoTitrate groups (e.g., GT HI NoTitrate vs. GT HI Titrate). The omnibus GLMM revealed significant Main effects only for Intake $F(1,89)=8.22$, $p=.0052$ but not for STNTGT $F(2,89)=.54$, $p>.10$ or Titration $F(1,89)=1.46$, $p>.10$. There were no significant two way interactions STNTGT by Titration $F(2,89)=2.10$, $p>.10$ or STNTGT by Intake $F(2,89)=1.25$, $p>.10$ or Intake by Titration $F(1,89)=.69$, $p>.10$. The three way interaction was also not significant STNTGT by Intake by Titration $F(2,89)=1.56$, $p>.10$.

Planned comparisons were made due to scientific interests in Titrate vs. NoTItrate groups and there was a significant difference in Uncued Response Rate between GT HI Titrate vs. GT HI NoTitrate ($p=.036$) but no other subgroup was significantly different ($p>.10$). These results provided further evidence that GT HI NoTitrate did not discriminate the tone. Furthermore, inspection of Figure 14 suggested that both ST HI Titrate and ST HI NoTitrate were equally poor at tone-discrimination (despite no group differences).

4.2.7— Video Analysis of Motor Behavior during Cocaine SA

Several analyses involved comparing FR during Hits vs. Misses, so it was important to identify if any neural differences were accounted for solely by locomotion

differences rather than other possible factors such as DL differences on Hits vs. Misses. Eliminating any motoric activity difference (before tone-onset) across Hits and Misses was important for preserving any putative neuronal correlations with the presence vs absence of a "readiness" state reflecting motivation to self-administer drug before the onset of the tone.

1. *Velocity on Hits vs. Misses*: A linear regression of velocity on Hits vs. velocity on Misses in R was significant $F(1,56)=45.84$, $p<.0001$, $R2=.45$, slope=.68, int=1.79, and this slope was not different than 1.0 ($p=.263$). These data indicate no difference in locomotor velocity on Hits vs. Misses one second prior to the onset of the tone-cue (Fig. 15). We then ran a repeated measures ANOVA in R where DV time 1 was equal to velocity on Hits and DV time 2 was equal to velocity on Misses and categorical IVs were STNTGT and Intake with posthoc analyses run on any significant main effects or interactions. Results indicated no Main effect of Hit vs. Miss $F(1,103)=.479$, $p>.10$, no interaction of Hit vs. Miss with any combination of STNTGT $F(2, 103)=.189$, $p>.10$ or Intake $F(1,103)=2.42$, $p>.10$ and the interaction with all three was also not significant $F(2, 103)=.102$, $p>.10$. Therefore, Hit vs. Miss velocity was not different overall, nor was velocity different within any individual subcategory.

2. Velocity on DL: It was important to identify any influence of DL on motoric behavior. A linear regression of velocity on Hits regressed on DL on Hits (Fig. 16) was significantly negatively correlated $F(1,56)=32.06$, $p<.0001$, slope=-.434, int=8.7 which was also the case for Misses $F(1, 56)=9.57$, $p=.003$, slope=-.29, int=6.91. These data suggest higher drug levels result in lower velocity for both Hits and for Misses.

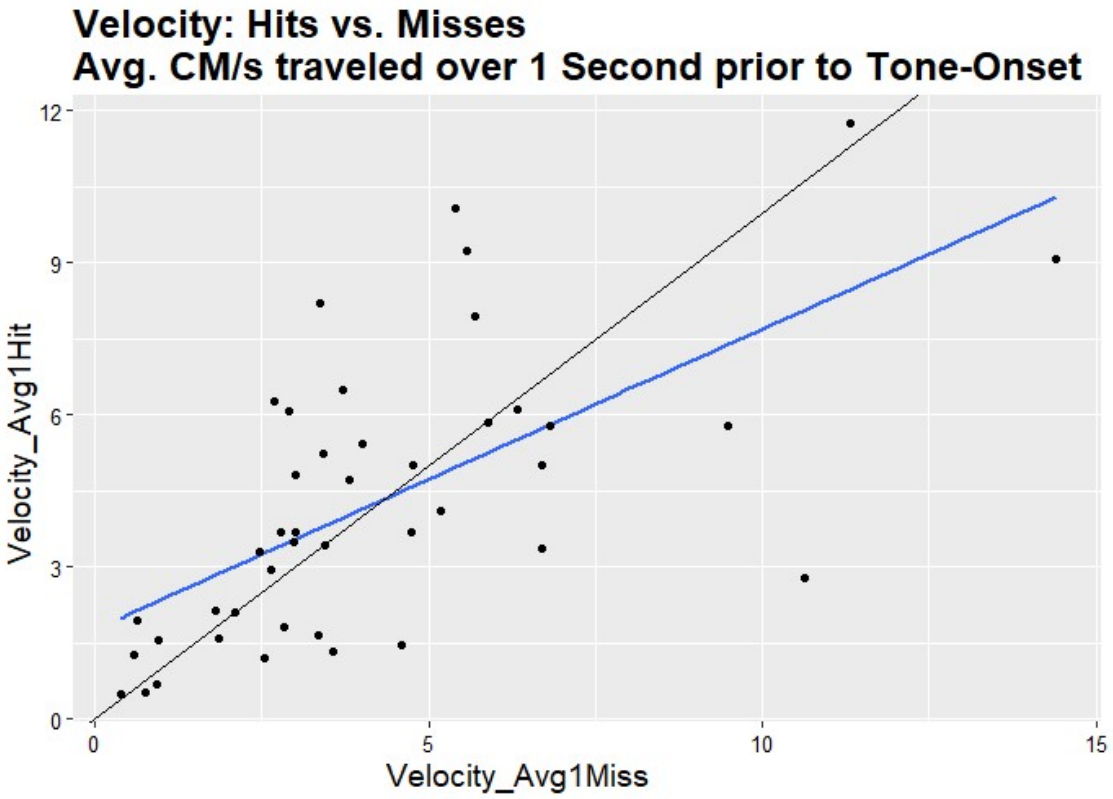

Figure 15: Velocity on hits (y axis) vs. velocity on misses (x axis), where each dot represents one session. Overall, velocity on Hits predicted velocity on Misses, and this slope was not different from 1.0. There was no difference in the speed of movement during the 1 second before tone onset on Hits vs. Misses.

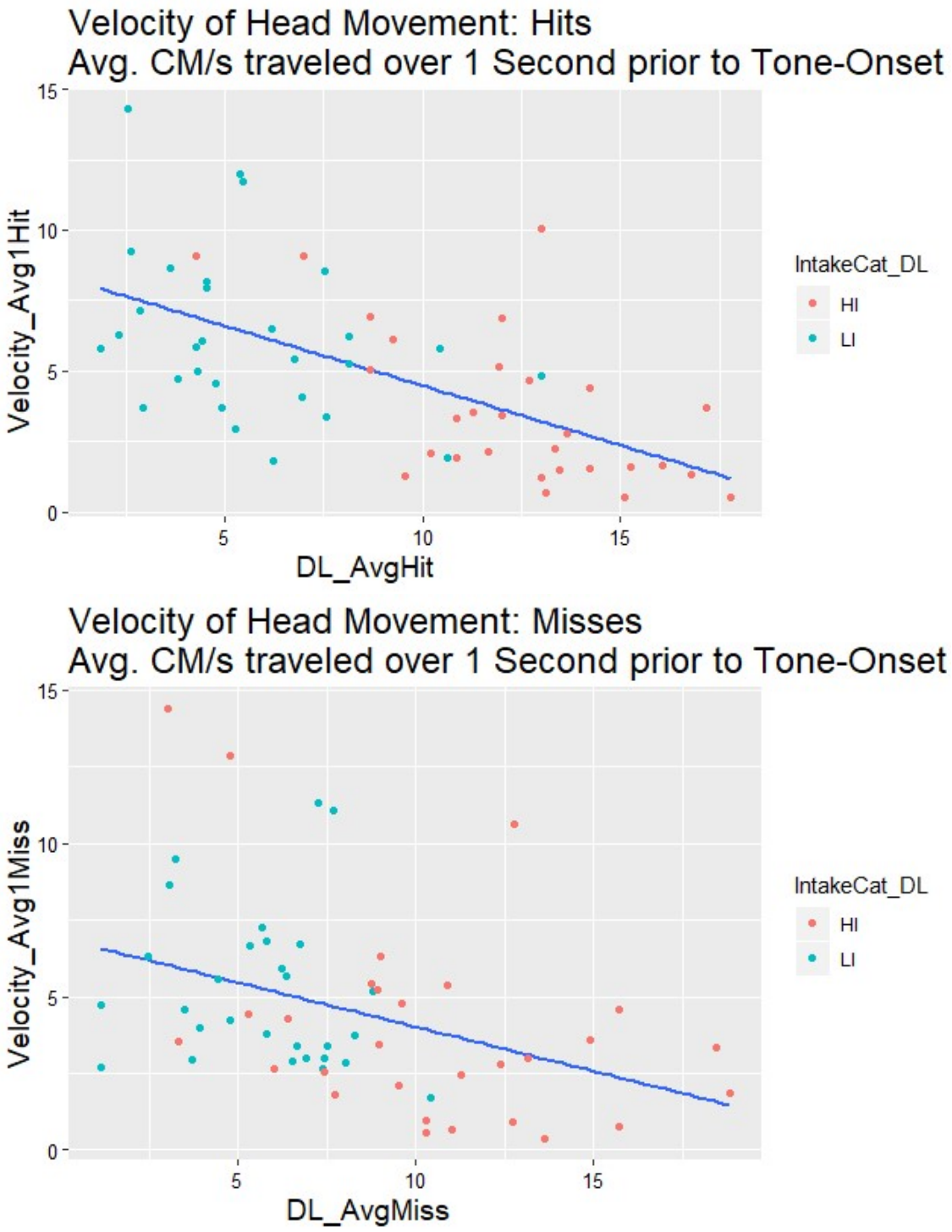

Figure 16: Velocity and DL: Each dot for these figures represent the average session velocity on Hits or Misses (Y axis) regressed on average session DL on Hits or Misses (X axis). For both Hits and Misses, velocity significantly decreased with DL which is consistent with literature suggesting animals transition from hyper-locomotive behavior at higher DL towards focused stereotypic head movements.

3. % of Session on DL: we also assessed the tendency of individual animals to remain near the operant corner as a function of increasing DL. A linear regression of % of Session in Q1 regressed on average session DL (Fig. 17) was significant $F(1,56)=29.95$, $p<.001$, slope=4.17, int=13.07. This finding suggests animals that self-administered higher brain level of cocaine remained at or around the operant corner for the majority of the session.

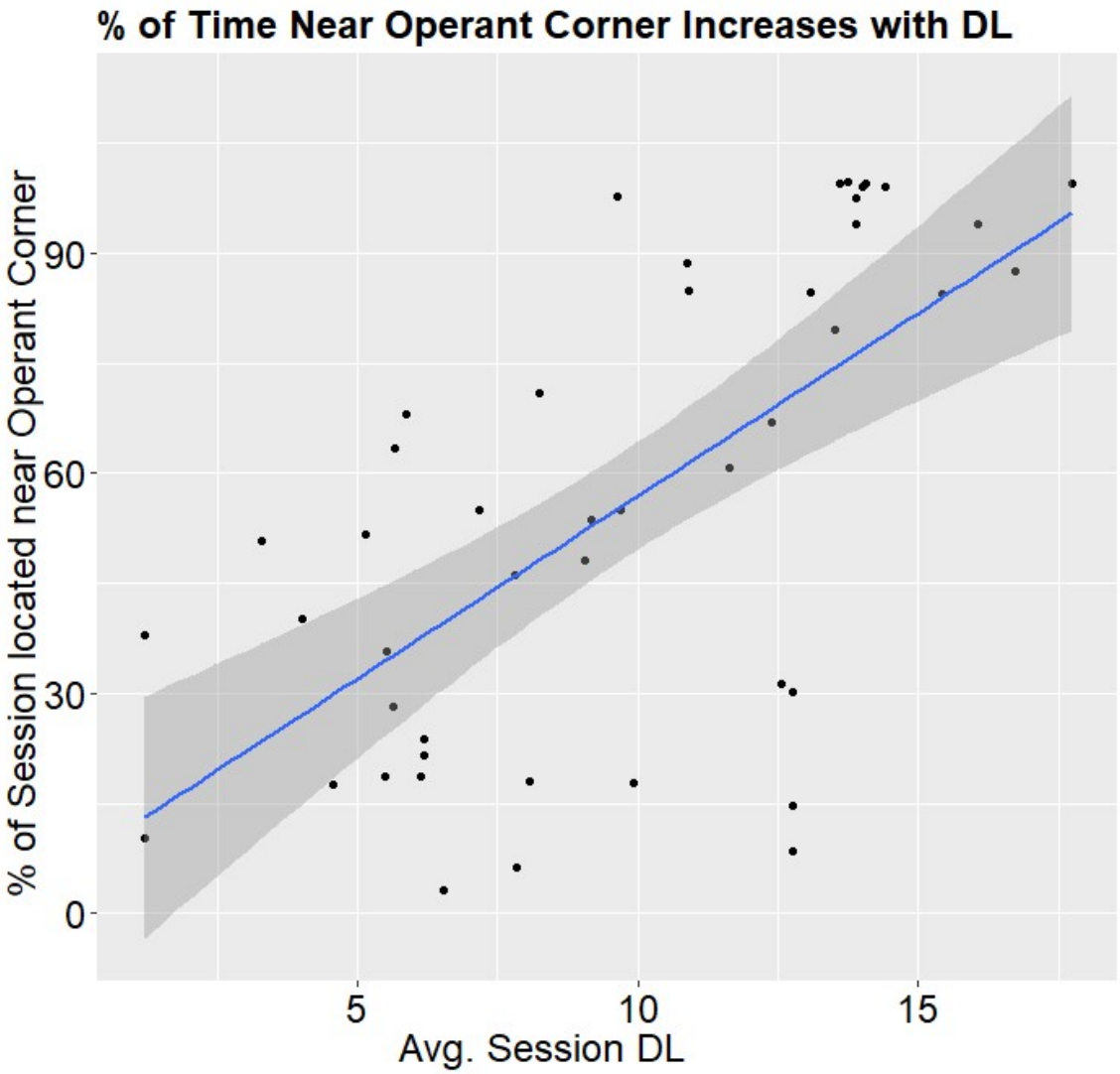

Figure 17: % of Session in Operant Corner increases with DL. One dot is a single session. The y axis represents the % of the total session the animal was tracked in Q1 (the operant corner) and the x axis is the avg. DL the animal maintained during the Maintenance phase. Subjects spent significantly greater proportions of the session in Q1 as session DL increased, suggesting the corner was highly salient in the highest intake subjects.

4.3— Tone-Evoked Processing:

4.3.1: Phase 1 "Tone-evoked FR": HI and LI, ST and GT and NT, Baseline vs. Posttone, Collapsed Response

Stability analyses were run separately for shell and core neurons were designed to address the following question: does the tone evoke a change in FR in any specific combination of Region, Intake Category, or STNTGT? Essentially, does the tone evoke a change in FR relative to Baseline FR?

Shell Phase 1: "Tone-evoked FR"

Although of less scientific importance than the planned a priori stability analyses, the omnibus LMM was a necessary first step in the process of generating the post hoc t-tests for the analysis of tone-evoked activity. The Main effects and Interactions are reported (with significance values in bold) in the table below:

Phase 1: LMM Omnibus Results for Shell Neurons				
Variables:	dF	N	F Value	P Value
Tone	**1**	**27774**	**133.30**	**<.0001**
STNTGT	**2**	**27774**	**4.71**	**.009**
Intake	1	27774	1.51	>.10
Tone * Intake	**1**	**27774**	**4.94**	**.0260**
STNTGT * Intake	**2**	**27774**	**4.15**	**.0026**
Tone * STNTGT	2	27774	2.06	>.10

Tone * Intake * STNTGT	**2**	**27774**	**5.97**	**.0026**

As previously mentioned the scientifically relevant test was on stability analysis of FR for individual categorical variables (complete results are included in Supplemental Table 1). We identified lack of "stability" (i.e., tone-evoked changes) in both GT HI, slope=1.490, 95% CI [1.170, 1.81], intercept=-.029 and GT LI, slope=.544, 95% CI [.325, .762], intercept=0.014 where both regression lines were outside the bounds of the line of no change (i.e., slopes were different from 1.0) (Fig. 18a). That is, because Baseline FR did not predict Posttone FR, such lack of stability was therefore considered evidence of tone-evoked activity in shell neurons. No other subcategory was significant and all others were "stable."

Core Phase 1

Although of less scientific importance than the planned a priori stability analyses, the omnibus LMM for core neurons was a necessary first step in the process of generating the post hoc t-tests for the analysis of tone-evoked activity. The Main effects and Interactions are reported (with significance values in bold) in the table below:

Phase 1: LMM Omnibus Results for Core Neurons				
Variables:	dF	N	F Value	P Value
Tone	**1**	**18031**	**34.64**	**<.0001**
STNTGT	2	18031	1.16	>.10
Intake	1	18031	1.04	>.10

Tone * Intake	1	18031	1.58	>.10
STNTGT * Intake	2	18031	.62	>.10
Tone * STNTGT	2	18031	1.04	>.10
Tone * Intake * STNTGT	2	18031	.82	>.10

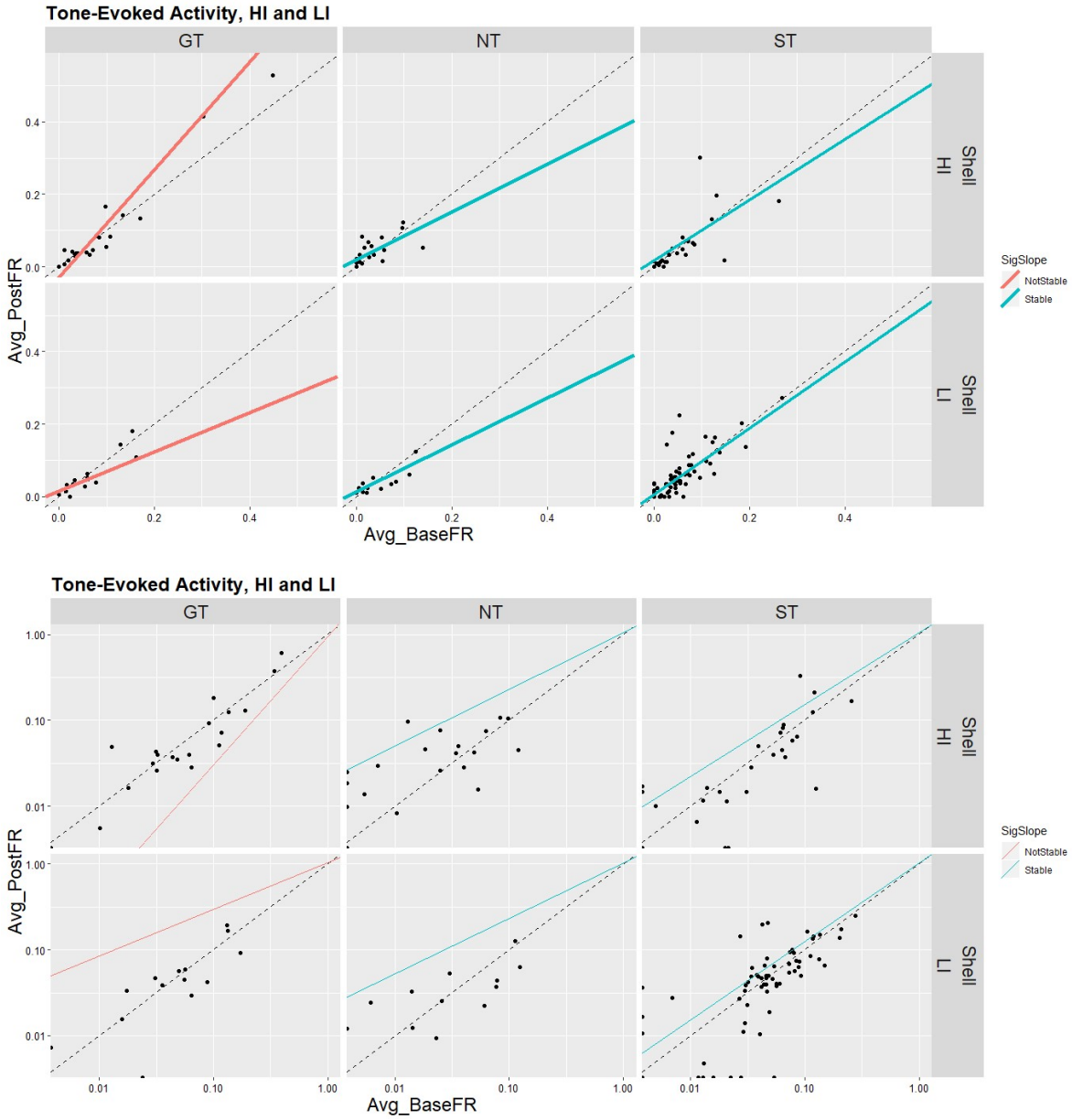

Figure 18a: Shell tone evoked changes for High Intake and Low Intake, ST and NT and GT, with Hits and Misses Collapsed. The x axis is the Mean Baseline FR and the y axis is the Mean Posttone FR for a particular Neuron from the specific subcategory of Intake, and STNTGT. Color of lines indicate if the slopes (Baseline vs. Posttone) were stable (Blue, Baseline vs. Posttone no different) or not stable (Red, Baseline vs. Posttone different, tone-evoked). The top figure represents raw FR scatterplots, and the bottom figure represents log scaled axes to enhance the location of individual neuron regressions. We identified lack of "stability" (i.e., tone-evoked changes) in both GT HI and GT LI where both regression lines were outside the bounds of the line of no change (i.e., slopes were different than 1.0) and were therefore considered evidence of tone-evoked activity. No other subcategory was significant and all others were considered "stable" (see supplemental table 1 for complete results).

As previously mentioned the scientifically relevant test was on stability analysis of FR for individual categorical variables. We identified lack of "stability" (i.e., tone-evoked changes) only for GT HI, slope=.261, 95% CI [-1.357, 1.880], intercept=.037 where the slope was not significantly different from 0 (p=.752) (Figure 18b). Therefore, GT HI neurons lacked "stability" and provided evidence of tone-evoked changes for core, as well as shell neurons. GT LI neurons showed tone-evoked changes in shell, but not core. All stability analysis results are indicated in Supplemental Table 1.

4.3.2: Phase 2 "Hit vs Miss": HI and LI, ST and GT and NT, Hit vs. Miss, Baseline vs. Posttone

Our Phase 2 stability analysis was designed to address the following question: does the tone evoke a change in FR for any specific combination of Region, Intake Category, or STNTGT during Hits or Misses? Furthermore, how is the tone selectively processed by Neurons during a Hit compared to a Miss (i.e., how do slopes differ across Response)? Finally, for any differences between Hits vs. Misses in tone-evoked change in FR (Posttone different from Baseline), is Baseline FR different?

Shell Phase 2

For purposes of generating the post hoc stability analyses the omnibus LMM for shell neurons are included in the table below:

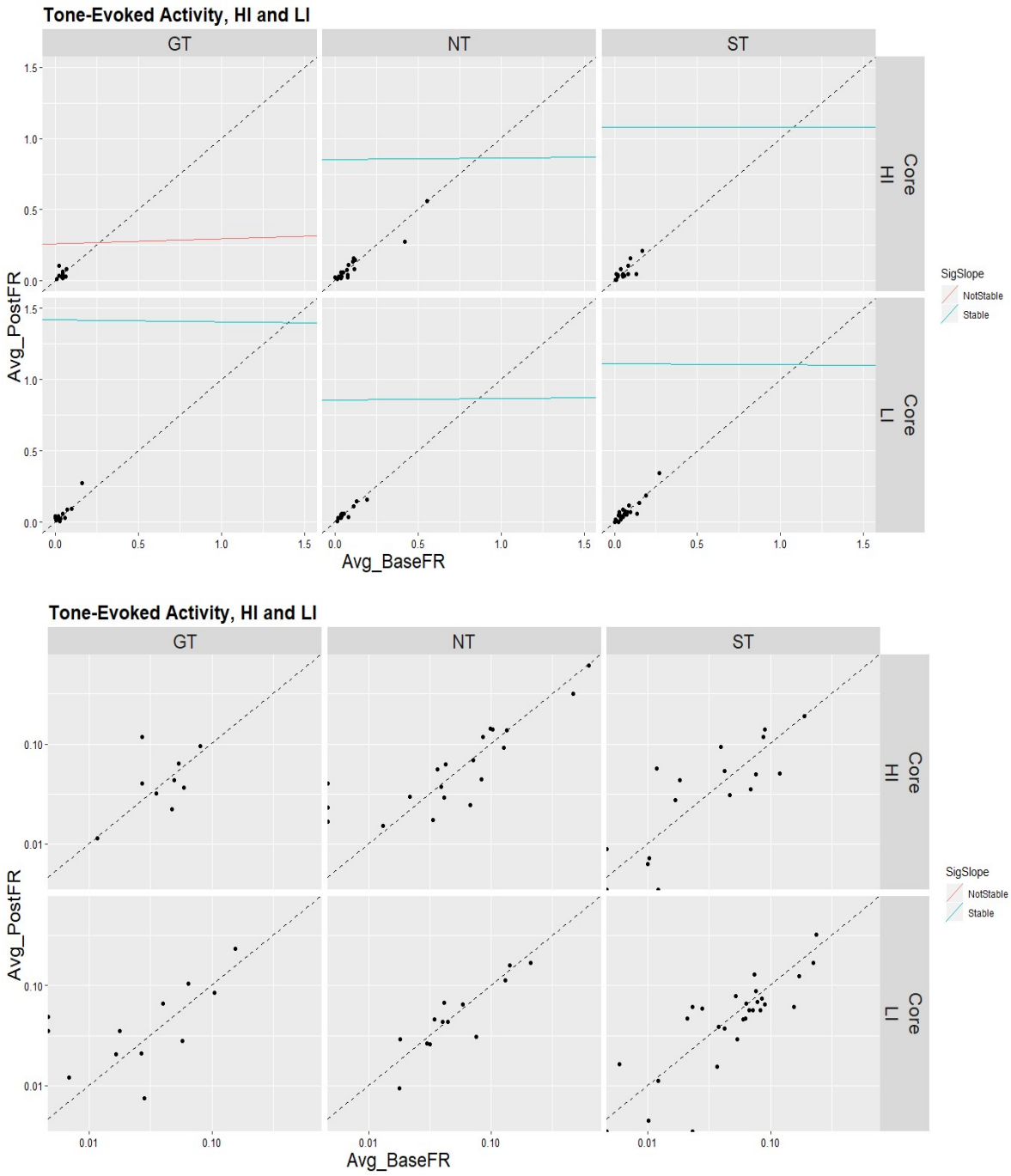

Figure 18b: Core tone evoked changes for High Intake and Low Intake, ST and NT and GT, with Hits and Misses Collapsed. The x axis is the Mean Baseline FR and the y axis is the Mean Posttone FR for a particular Neuron from the specific subcategory of Intake, and STNTGT. Color of lines indicate if the slopes (Baseline vs. Posttone) were stable (Blue, Baseline vs. Posttone no different) or not stable (Red, Baseline vs. Posttone different, tone-evoked). The top figure represents raw FR scatterplots, and the bottom figure represents log scaled axes to enhance the

location of individual neuron regressions. We identified lack of “stability” (i.e., tone-evoked changes) only for GT HI, where the slope was not significantly different than 0 (p=.752). See supplemental table 1 for complete results.

Phase 2: LMM Omnibus Results for Shell Neurons				
Variables:	dF	N	F Value	P Value
Tone	**1**	**27774**	**34.64**	**<.0001**
Response (Hit vs. Miss)	**1**	**27774**	**4.25**	**.0392**
STNTGT	2	27774	.68	>.10
Intake	1	27774	.07	>.10
Tone * Response	**1**	**27774**	**7.42**	**.0065**
Tone * Intake	1	27774	1.02	>.10
STNTGT * Intake	2	27774	1.30	>.10
STNTGT * Response	2	27774	2.56	.078
Response * Intake	1	27774	.61	>.10
Tone * STNTGT	2	27774	.96	>.10
STNTGT * Response * Intake	**2**	**27774**	**4.00**	**.0183**
Tone * STNTGT * Response	2	27774	1.22	>.10
Tone * Intake * STNTGT	2	27774	2.58	.076
Tone * Response * Intake	1	27774	1.19	>.10
Tone * Response * Intake * STNTGT	2	27774	1.61	>.10

The analysis of tone-evoked activity, i.e., stability, was first analyzed for Hits and Misses separately among categorical variables (HI and LI, ST and NT and GT). All stability analysis results are indicated in Supplemental Table 2a, and graphically represented in Figure 19a. Tone-evoked changes were present for all ST, NT and GT HI shell Neurons during Misses (but not

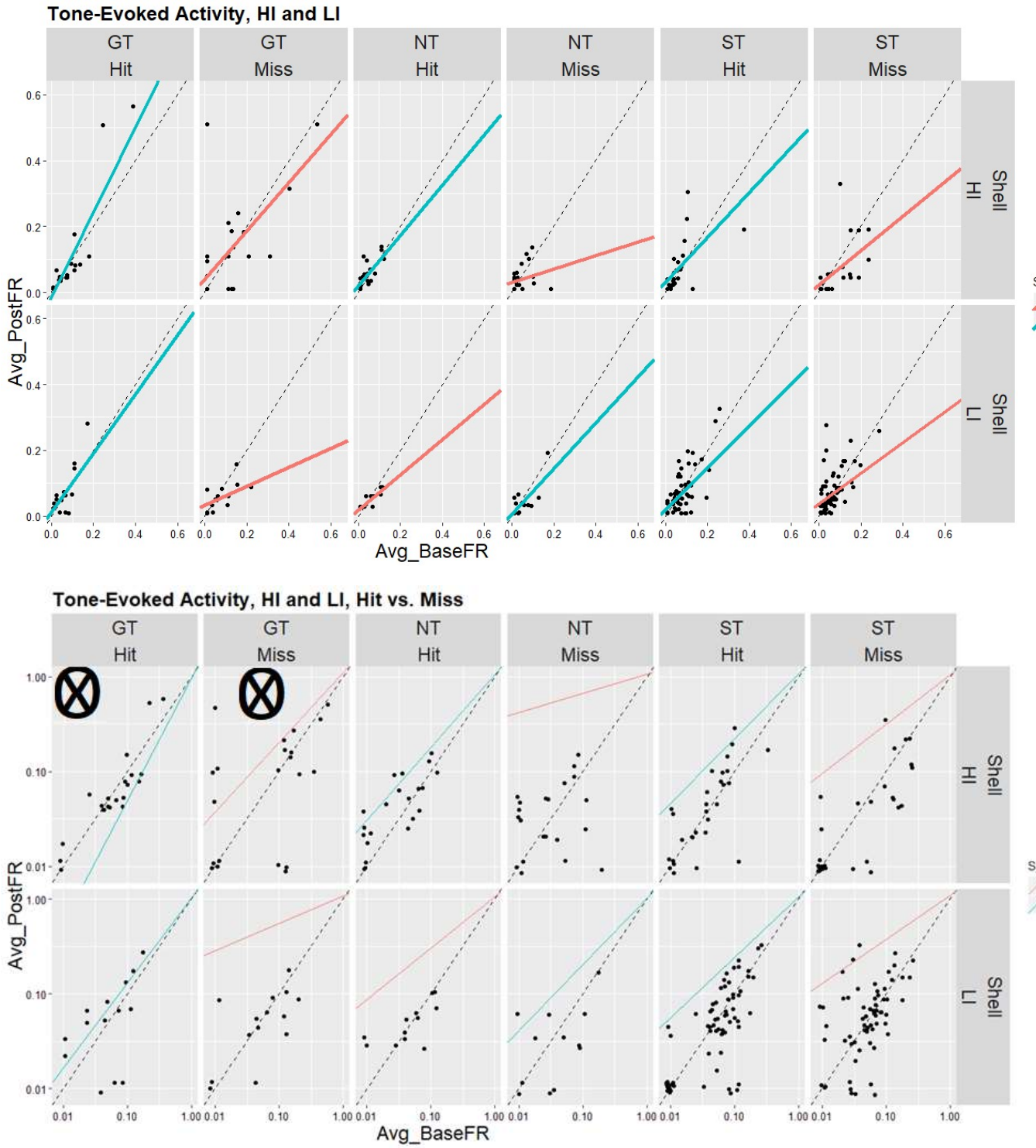

Figure 19a: tone evoked changes for Shell, High Intake and Low Intake, ST and NT and GT, with Hits and Misses separated. The x axis is the Mean Baseline FR and the y axis is the Mean Posttone FR for a particular Neuron from the specific subcategory of Intake, STNTGT, and Response. Color of lines indicate if the slopes (Baseline vs. Posttone) were stable (Blue, Baseline vs. Posttone no different) or not stable (Red, Baseline vs. Posttone

different, tone-evoked). The top figure represents raw FR scatterplots, and the bottom figure represents log scaled axes to enhance the location of individual neuron regressions. The “X” in the top left signals a significant difference in slopes between Hits vs. Misses, an open circle “O” in the top left signals a significant difference in Average Baseline FRs between Hits vs. Misses, and a merged x and open circle “⊗” indicates both (Hit vs. Miss slope differences, and Hit vs. Miss baseline differences). As indicated above by colors: Tone-evoked changes were present for all HI Neurons during Misses (but not Hits). For LI neurons, tone evoked changes were present for GT LI Misses, ST LI Misses, and NT LI Hits. Results suggest evidence of tone processing on Misses among all HI groups and 2/3 of LI groups. As indicated above by symbols: GT HI was the only subcategory to show differential tone processing on Hits vs. Misses, and also showed Baseline FR differences on Hits vs. Misses (p=.036) however, baseline differences do not appear to account for any dramatic shift in regression lines. It is also important to note that comparisons across Response are from the same neuron. See Supplemental Table 2a for complete results.

Hits): ST HI Miss slope=.522, 95% CI [0.312, 0.731], intercept=.021, GT HI Miss slope=0.735, 95% CI [0.549, .922), intercept=.039 and NT HI Miss slope= 0.205, 95% CI [-0.242, 0.653], intercept=.030, p=.369 (not different vs. 0). For LI neurons, ST LI Miss slope=.4657, 95% CI [0.227, 0.704], intercept=.036 and GT LI Miss slope=0.290, 95% CI [-0.066, 0.646], intercept=.032, p=.111 (not different vs. 0) and NT LI Hits slope=0.538, 95% CI [0.234, 0.843], intercept=.021. Results provide evidence of tone processing on Misses by shell neurons in all HI groups and 2/3 of LI groups.

Slopes were compared for any categorical variable that demonstrated tone-evoked changes on either Hits or Misses. GT HI was the only subcategory to show differential tone processing by shell neurons on Hits vs. Misses, $Diff_{Slopes}$= 0.556, t(27622)=3.05, p=0.014. No other subcategory showed Hit vs. Miss differences in slopes GT LI $Diff_{Slopes}$= 0.606, t(27622)=1.62, p>.10, NT HI $Diff_{Slopes}$= 0.561, t(27622)=2.13, p>.10, NT LI $Diff_{Slopes}$= -0.157, t(27622)=-.570, p>.10, ST HI $Diff_{Slopes}$= 0.162, t(27622)=.550, p>.10, and ST LI $Diff_{Slopes}$= 0.1722, t(27622)=.209, p>.10.

We then tested mean baseline FR differences in Hit vs. Misses. For purposes of completeness the omnibus GLMM are included in the table below:

Phase 2: Baseline FR Comparisons, LMM Omnibus Results for Shell Neurons				
Variables:	dF	N	F Value	P Value
Response	**1**	27634	**6.30**	**.012**
STNTGT	**2**	**27634**	**4.41**	**.012**
Intake	1	27634	.82	>.10

Response * Intake	1	18031	2.84	.092
STNTGT * Intake	2	18031	1.20	>.10
Response * STNTGT	2	18031	3.27	.038
Response * Intake * STNTGT	2	18031	.90	>.10

Post hoc analyses revealed baseline FR differences only for GT HI (p=.036) but no other group showed baseline FR differences (p>.10). Results indicate tone-processing on Misses for all HI groups, and only GT HI tone processing was significantly different on Hits vs. Misses.

Core Phase 2

For purposes of generating the post hoc stability analyses the omnibus LMM are revealed in the table below:

Phase 2: LMM Omnibus Results for Core Neurons				
Variables:	dF	N	F Value	P Value
Tone	**1**	18031	**53.31**	**<.0001**
Response	1	18031	1.18	.09
STNTGT	2	18031	.58	>.10
Intake	1	18031	1.07	>.10
Tone * Response	1	18031	2.25	>.10

Tone * Intake	1	18031	1.13	>.10
STNTGT * Intake	2	18031	.63	>.10
STNTGT * Response	2	18031	2.10	.078
Response * Intake	1	18031	.48	>.10
Tone * STNTGT	2	18031	.18	>.10
STNTGT * Response * Intake	2	18031	.96	>.10
Tone * STNTGT * Response	**2**	**18031**	**7.76**	**.0004**
Tone * Intake * STNTGT	2	18031	1.31	>.10
Tone * Response * Intake	1	18031	.45	>.10
Tone * Response * Intake * STNTGT	**2**	**18031**	**4.53**	**.0108**

The analysis of tone-evoked activity was first analyzed for Hits and Misses separately among categorical variables (HI and LI, ST and NT and GT). All stability analysis results are indicated in Supplemental Table 2b and graphically represented in Figure 19b. Tone-evoked changes were not present at all for HI or LI ST core neurons on either Hits or Misses. Tone evoked changes were present among HI neurons for GT HI Hits slope=0.434, 95% CI [-0.496, 1.364], intercept=.026, p=.36 (not different vs. 0) and GT HI Miss slope=.056, 95% CI [-1.040, 1.152], intercept=.081, p=.92 (not different vs. 0) and NT HI Misses (but not Hits), slope= 0.858, 95% CI [0.741, 0.975], intercept=0.021. For LI neurons, tone-evoked changes were present only in GT LI Miss slope=.484, 95% CI [.372, 0.597], intercept=0.023 and for NT LI Hits slope=0.412, 95% CI [.131, 0.694], intercept=0.02. Results suggest evidence of tone processing to some degree by core neurons in all groups except ST, and tone processing during Hits and Misses for HI GT.

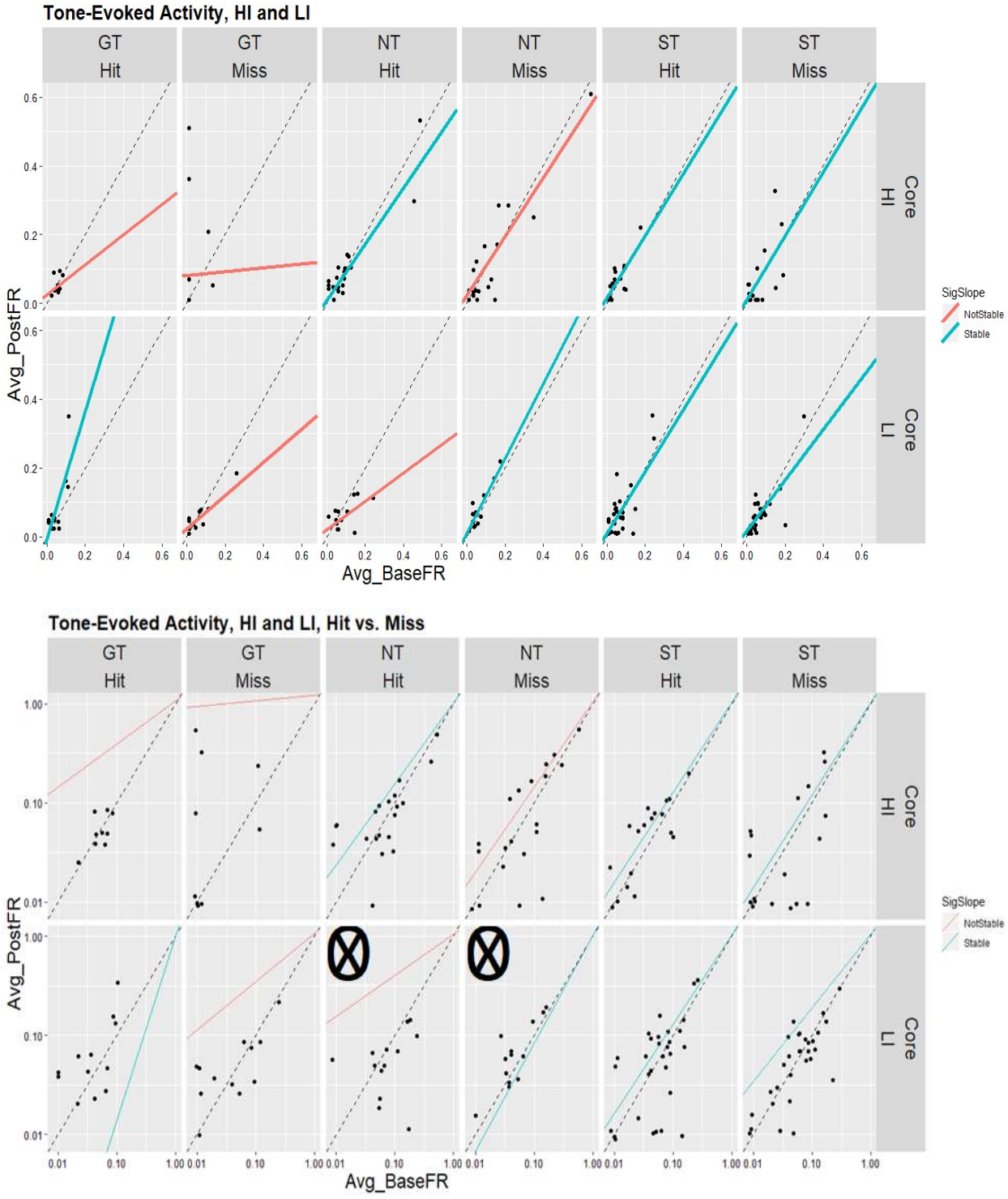

Figure 19b: tone evoked changes for core, High Intake and Low Intake, ST and NT and GT, with Hits and Misses separated. The x axis is the Mean Baseline FR and the y axis is the Mean Posttone FR for a particular Neuron from the specific subcategory of Intake, STNTGT, and Response. Color of lines indicate if the slopes (Baseline vs. Posttone) were stable (Blue, Baseline vs. Posttone no different) or not stable (Red, Baseline vs. Posttone

different, tone-evoked). The top figure represents raw FR scatterplots, and the bottom figure represents log scaled axes to enhance the location of individual neuron regressions. The “X” in the top left signals a significant difference in slopes between Hits vs. Misses, an open circle “O” in the top left signals a significant difference in Average Baseline FRs between Hits vs. Misses, and a merged x and open circle “⊗” indicates both (Hit vs. Miss slope differences, and Hit vs. Miss baseline differences). As indicated above by colors: Tone-evoked changes were not present at all for HI or LI ST groups for either Hits or Misses. Tone evoked changes were present among HI neurons for GT HI Hits, GT HI Misses, and NT HI Misses. For LI neurons, tone-evoked changes were only present in GT LI Misses, and NT LI Hits. Results suggest evidence of tone-evoked processing to some degree in all groups except ST, and processing during both Hits and Misses for GT HI. As indicated above by the symbols: NT LI was the only subcategory to show differential tone processing on Hits vs. Misses ($p=.0004$) and also Hit vs. Miss Baseline FR differences ($p=.001$). See Supplemental Table 2b for complete results.

Slopes were then compared for any categorical variable that demonstrated tone-evoked changes on either Hits or Misses. NT LI was the only subcategory to show differential tone processing on Hits vs. Misses, $Diff_{Slopes}$= -0.676, t(17925)= -4.01, p=.0004. No other subcategory showed Hit vs. Miss differences in tone-processing: GT HI $Diff_{Slopes}$= 0.378, t(17925)= 1.01, p>.10. GT LI $Diff_{Slopes}$= 1.37, t(17925)= 2.25, p>.10, NT HI $Diff_{Slopes}$=-.043, t(17925)= -.38, p>.10. Results indicate that tone-processing was present in HI GT core neurons, but Hit and Miss slopes were not different.

We then tested mean baseline FR differences in core neurons on Hits vs. Misses. For purposes of completeness the omnibus GLMM results are included below:

Phase 2: Baseline FR Comparisons, LMM Omnibus Results for Core Neurons				
Variables:	dF	N	F Value	P Value
Response	1	17937	.27	>.10
STNTGT	2	17937	1.73	>.10
Intake	1	17937	1.73	>.10
Response * Intake	1	17937	1.09	>.10
STNTGT * Intake	2	17937	.26	>.10
Response * STNTGT	2	17937	1.49	>.10
Response * Intake * STNTGT	2	17937	2.70	>.10

Post hoc analyses did reveal baseline FR differences for NT LI (p=.0011) but no other group showed baseline FR differences in core neurons (p>.10).

4.3.3: Phase 3 "Titrate and NoTitrate": HI and LI, ST and GT and NT, Hit vs. Miss, Baseline vs. Posttone

Phase 3 of the stability analysis was designed to address the following question: does the tone evoke a change in FR in any specific combination of Region, Intake Category, Phenotype, or Titration (Titrate or NoTitrate) during Hits or Misses? How is the tone selectively processed by the neuron when a Hit response was the outcome compared to a Miss? The inclusion of Titration as a factor was important to include because high cocaine level can suppress NAc activity in a majority of neurons (Peoples & West, 1996; Nicola & Deadwyler, 2000). By definition (operationalized in section 3.3, equation 4), DL was lower on Hits than Misses during Titration, but higher on Hits than Misses during Non-Titration. We also ran separate analyses for HI and LI groups due to our interest in how HI (i.e., potential high risk) groups processed the tone on days when DL could not be "controlled" (i.e., NoTitrate). Do neurons process the tone differently when the sessions resulted in Titrate?

High Intake Shell Phase 3

We first ran the same omnibus LMM for HI categories separately from LI Categories. For purposes of generating the post hoc stability analyses for HI the omnibus LMM are reported in the table below:

Phase 3: LMM Omnibus Results for High Intake, Shell Neurons				
Variables:	dF	N	F Value	P Value

Tone	**1**	11144	32.69	**<.0001**
Response	**1**	**11144**	**3.89**	**.049**
STNTGT	2	11144	.57	>.10
Titration	1	11144	1.41	>.10
Tone * Response	**1**	**11144**	**10.14**	**.0015**
Tone * Titration	1	11144	.11	>.10
STNTGT * Titration	**2**	**11144**	**6.16**	**.0021**
STNTGT * Response	2	11144	.88	>.10
Response * Titration	1	11144	.94	>.10
Tone * STNTGT	2	11144	1.19	>.10
STNTGT * Response * Titration	2	11144	1.92	>.10
Tone * STNTGT * Response	2	11144	1.24	>.10
Tone * Titration * STNTGT	**2**	**11144**	**5.28**	**.0051**
Tone * Response * Titration	1	11144	3.49	.06
Tone * Response * Titration * STNTGT	2	11144	.79	>.10

The analysis of tone-evoked activity was first analyzed for Hits and Misses separately among HI categorical variables (Titrate and NoTitrate, ST and NT and GT). All stability analysis results are indicated in Supplemental Table 3a1 and graphically represented in Figure 20a. Tone-evoked changes were present for all HI shell Neurons regardless of Titrate or NoTitrate during Misses: ST HI Miss NoTitrate slope=0.494, 95% CI [0.278, 0.710], intercept=.015, NT HI Miss NoTitrate slope=0.805, 95% CI [-0.168,

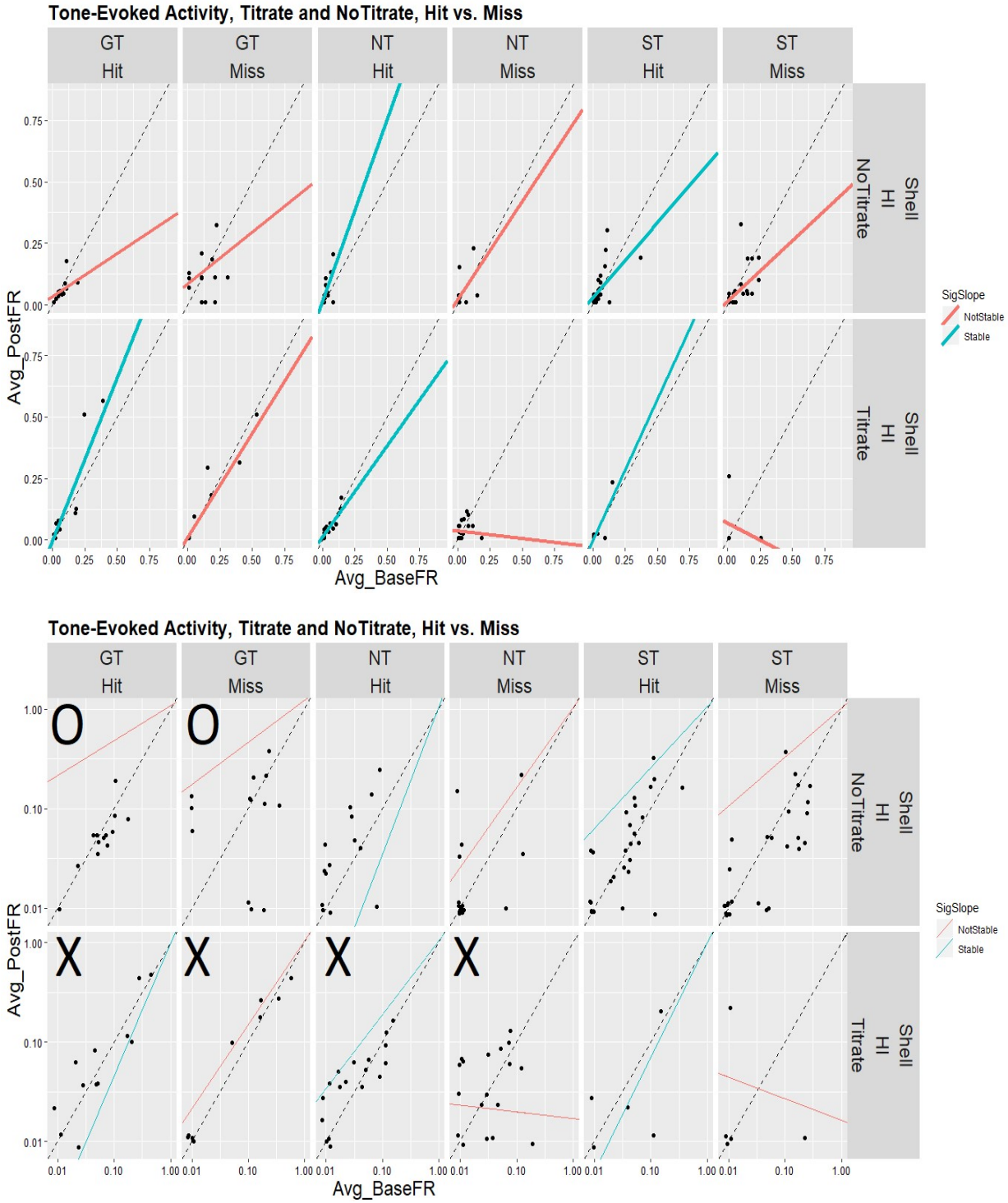

Figure 20a: tone evoked changes for Shell, High Intake, ST and NT and GT, Titrate and NoTitrate, with Hits and Misses separated. The x axis is the Mean Baseline FR and the y axis is the Mean Posttone FR for a particular Neuron from the specific subcategory of STNTGT, Titration, and Response. Color of lines indicate if the slopes (Baseline vs. Posttone) were stable (Blue, Baseline vs. Posttone no different) or not stable (Red, Baseline vs. Posttone different, tone-evoked). The top figure represents raw FR scatterplots, and the bottom figure represents log scaled axes to

enhance the location of individual neuron regressions. The “X” in the top left signals a significant difference in slopes between Hits vs. Misses, an open circle “O” in the top left signals a significant difference in Average Baseline FRs between Hits vs. Misses, and a merged x and open circle “⊗” indicates both (Hit vs. Miss slope differences, and Hit vs. Miss baseline differences). As indicated above by colors: Results suggest Misses had tone-evoked changes among all HI shell Titrate and NoTitrate groups, while tone-processing on Hits was only evident among HI GT NoTitrate neurons. As indicated above by symbols: only HI Titrate shell neurons processed the tone differentially on Hits vs. Misses, where GT HI Titrate showed differential tone processing on Hits vs. Misses, as did NT HI Titrate, but not ST HI. Mean Baseline FR Hit vs. Miss differences were found for GT HI NoTitrate shell ($p=.021$) but inspection of the figure indicates differences n baseline activity was not responsible for “masking” underlying tone-evoked processing differences. (see Supplemental Table 3a1 for complete results). Thus, when HI groups Titrated (except ST) there was a difference in tone-evoked activity for Hits vs. Misses, but there was no difference in tone-evoked activity across Response for any “NoTitrate” groups.

1.778], intercept=.018, p=.105 (not different vs. 0), GT HI Miss NoTitrate slope=0.4206, 95% CI [-0.170, 1.011], intercept=.086, p=.163 (not different vs. 0), ST HI Miss Titrate slope= -0.274, 95% CI [-0.640, 0.093], intercept=.067, p=.1432 (not different vs. 0), NT HI Miss Titrate slope= -0.065, 95% CI [-0.342, 0.213], intercept=.038, p=0.648 (not different vs. 0), GT HI Miss Titrate slope=0.8429, 95% CI [0.694, 0.992] intercept=.012. The only evidence of tone-evoked changes during Hits was unique to GT HI NoTitrate slope=0.349, 95% CI [-0.205, 0.902], intercept=.034, p=.217 (not different vs. 0). Results indicate strong evidence of tone-evoked changes on Misses universally among HI shell Titrate and NoTitrate groups, while tone-processing on Hits was evident only among HI GT NoTitrate shell neurons.

Slopes were then compared for any categorical variable that demonstrated tone-evoked changes on either Hits or Misses. Results suggested only HI Titrate shell neurons processed the tone differentially on Hits vs. Misses, where GT HI Titrate showed differential tone processing on Hits vs. Misses, $Diff_{Slopes}$= 0.486, t(11055)=2.72, p=0.033 as did NT HI Titrate $Diff_{Slopes}$= 0.804, t(11055)=4.20, p=0.0002 but not ST HI Titrate $Diff_{Slopes}$= 1.43, t(11055)=2.07, p>.10. There was no evidence of differential Hit vs. Miss slopes for any NoTitrate category; ST HI NoTitrate $Diff_{Slopes}$= .12, t(11055)=.45, p>.10 and NT HI NoTitrate $Diff_{Slopes}$= .67, t(11055)=.122, p>.10 and GT HI NoTitrate $Diff_{Slopes}$= -.07, t(11055)=-.15, p>.10 were all not significant.

We then tested mean baseline FR differences in Hit vs. Misses for every subcategory of HI shell neurons. For purposes of completeness the omnibus GLMM is included below:

Phase 3: Baseline FR Comparisons, LMM Omnibus Results for HI Shell Neurons				
Variables:	dF	N	F Value	P Value
Response	1	11230	2.61	>.10
STNTGT	**2**	**11230**	**4.39**	**.012**
Titration	1	11230	.12	>.10
Response * Titration	1	11230	1.37	>.10
STNTGT * Titration	2	11230	.47	>.10
Response * STNTGT	2	11230	1.74	>.10
Response * Titration * STNTGT	2	11230	.09	>.10

Post hoc analyses revealed baseline FR differences between Hits vs. Misses only for GT HI NoTitrate (p=.021) but no other group showed baseline FR differences in shell neurons (p>.10).

Thus, for HI shell neurons the tone was universally processed on Misses for every Titrate and NoTitrate group. Furthermore, there was differential processing of the tone across Hits and Misses only in groups that Titrated except for ST HI. There also was no observed difference in Hit vs. Miss Baseline FR for any Titrate group. The only group that demonstrated tone-evoked changes on Hits was GT HI NoTitrate, but this was not different from Miss tone-processing. Baseline FR differences on Hits vs. Misses did not influence the directionality of Hit or Miss slopes.

High Intake Core Phase 3

For purposes of generating the post hoc stability analyses for only HI core groups the omnibus LMM are included below:

Phase 3: LMM Omnibus Results for High Intake, Core Neurons				
Variables:	dF	N	F Value	P Value
Tone	**1**	7782	27.99	**<.0001**
Response	1	7782	.05	>.10
STNTGT	2	7782	.89	>.10
Titration	1	7782	.76	>.10
Tone * Response	1	7782	1.17	>.10
Tone * Titration	1	7782	1.56	>.10
STNTGT * Titration	2	7782	.38	>.10
STNTGT * Response	2	7782	2.42	.09
Response * Titration	1	7782	3.11	.08
Tone * STNTGT	**2**	**7782**	**3.11**	**.045**
STNTGT * Response * Titration	2	7782	.35	>.10
Tone * STNTGT * Response	2	7782	1.89	>.10
Tone * Titration * STNTGT	**2**	7782	**5.65**	**.005**
Tone * Response * Titration	1	7782	.01	>.10
Tone * Response * Titration * STNTGT	**2**	**7782**	**3.25**	**.039**

Tone-evoked activity was first analyzed for Hits and Misses separately among just HI core categorical variables (Titrate and NoTitrate, ST and NT and GT). All stability analysis results are indicated in Supplemental Table 3b1 and graphically represented in Figure 20b. A major finding appeared for core neurons: tone-evoked changes were present in almost every possible combination of Hits and Misses for Titrate and NoTitrate HI core neurons: ST HI Miss NoTitrate slope=0.6005, 95% CI [-0.0342, 1.235], intercept= 0.014, p=.067 (not different vs. 0), NT HI Miss

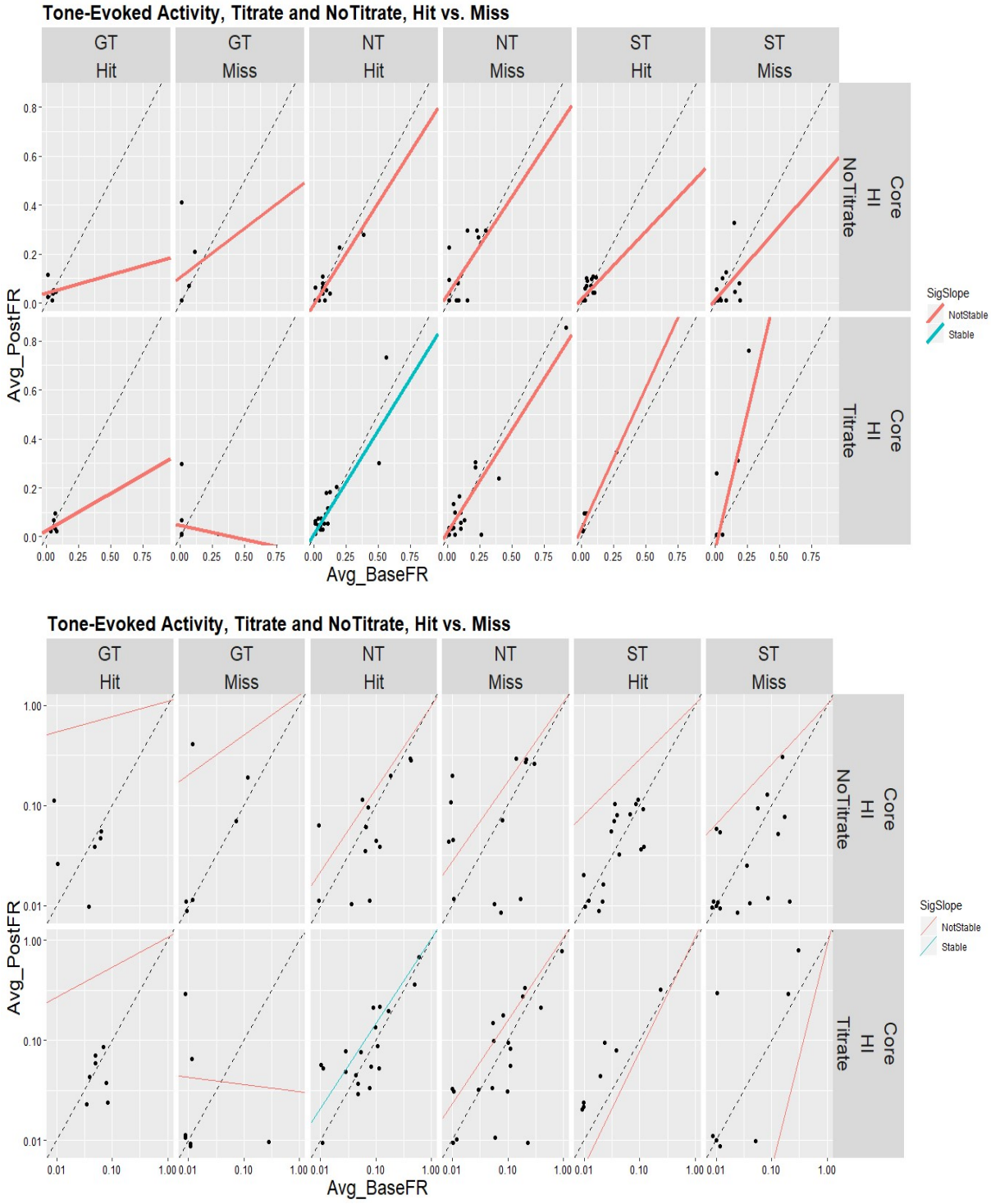

Figure 20b: tone evoked changes for core, High Intake, ST and NT and GT, Titrate and NoTitrate, with Hits and Misses separated. The x axis is the Mean Baseline FR and the y axis is the Mean Posttone FR for a particular Neuron from the specific subcategory of STNTGT, Titration, and Response. Color of lines indicate if the slopes (Baseline vs. Posttone) were stable (Blue, Baseline vs. Posttone no different) or not stable (Red,

Baseline vs. Posttone different, tone-evoked). The top figure represents raw FR scatterplots, and the bottom figure represents log scaled axes to enhance the location of individual neuron regressions. The “X” in the top left signals a significant difference in slopes between Hits vs. Misses, an open circle “O” in the top left signals a significant difference in Average Baseline FRs between Hits vs. Misses, and a merged x and open circle “⊗” indicates both (Hit vs. Miss slope differences, and Hit vs. Miss baseline differences). As indicated above by colors: A major finding appeared for core neurons where tone-evoked changes were present in almost every possible combination of Hits and Misses for Titrate and NoTitrate HI core neurons. The only subcategory that was not “tone-evoked” was NT HI Hit Titrate. Results suggest tone-evoked changes were present for almost every possible category on Hits and Misses, however, as indicated above by the lack of symbols, the results suggested an almost complete lack of evidence of tone-evoked differences on Hits vs. Misses. There was no evidence of Baseline FR differences present in Hits vs. Misses for any category (see Supplemental Table 3b1 for details).

NoTitrate slope=0.8049, 95% CI [0.602, 0.994], intercept= 0.035, GT HI Miss NoTitrate slope=0.3999, 95% CI [-1.548, 2.348], intercept= 0.104, p=.687 (not different vs. 0), ST HI Hit NoTitrate slope=0.5531, 95% CI [0.3172, 0.7890], intercept= 0.016, NT HI Hit NoTitrate slope=0.8285, 95% CI [0.676, 0.981], intercept= -0.004, GT HI Hit NoTitrate slope=.1512, 95% CI [-0.423, 0.725], intercept= 0.040, p= .606 (not different vs. 0), ST HI Miss Titrate slope=2.2197, 95% CI [1.307, 3.133], intercept= -0.046, NT HI Miss Titrate slope=0.8298, 95% CI [0.687, 0.973], intercept= 0.022, GT HI Miss Titrate slope=-0.1165, 95% CI [-0.339, 0.106], intercept= 0.047, p= .304 (not different vs. 0), ST HI Hit Titrate slope= 1.1459, 95% CI [1.054, 1.238], intercept= 0.036, GT HI Hit Titrate slope=0.2999, 95% CI [-0.5504, 1.150], intercept= 0.027, p= .489 (not different vs. 0). The only subcategory that did not exhibit "tone-evoked" core firing was NT HI Hit Titrate slope=0.8460, 95% CI [0.373, 1.319], intercept= 0.013. Therefore, results suggest tone-evoked changes were present for almost every possible category on Hits and Misses.

Slopes were then compared for any category hat demonstrated tone-evoked changes on Hits or Misses (i.e., every category for HI core neurons). Results suggested a complete lack of evidence of tone-evoked differences on Hits vs. Misses: GT HI Titrate $Diff_{Slopes}$= 0.416, t(7853)= 1.01, p>.10, GT HI NoTitrate $Diff_{Slopes}$= -0.249, t(7853)= -.29, p>.10, ST HI Titrate $Diff_{Slopes}$= -1.07, t(7853)= -2.46, p= 0.0797, NT HI Titrate $Diff_{Slopes}$= 0.016, t(7853)= .09, p>.10, NT HI NoTitrate $Diff_{Slopes}$= 0.030, t(7853)= .25, p>.10, ST HI NoTitrate $Diff_{Slopes}$= 0.047, t(7853)= -.18, p>.10.

We then tested mean baseline FR in Hit vs. Misses for every subcategory of HI core neurons. For purposes of completeness the omnibus GLMM is reported in the table below:

Phase 3: Baseline FR Comparisons, LMM Omnibus Results for HI Core Neurons				
Variables:	dF	N	F Value	P Value
Response	1	7865	.05	>.10
STNTGT	2	7865	1.75	>.10
Titration	**1**	**7865**	**4.27**	**.039**
Response * Titration	1	7865	.41	>.10
STNTGT * Titration	2	7865	.41	>.10
Response * STNTGT	2	7865	.84	>.10
Response * Titration * STNTGT	2	7865	.36	>.10

Post hoc analyses revealed no baseline FR differences between Hits vs. Misses for any subcategory of HI core neurons ($p>.10$).

Thus, for HI core neurons the tone-evoked a change in FR in almost every possible combination of HI core STNTGT, Titration, and Response except for HI NT Hits. However, the tone was processed equivalently on Hits and Misses for all subcategories and there was no influence of baseline FR differences for any category across Hits and Misses.

Low Intake Shell Phase 3

We ran the same omnibus LMM for LI categories separately from HI Categories. It should also be noted that GT LI always Titrated and therefore we could not analyze NoTitrate neurons in either core or shell LI GT. For purposes of generating the post hoc stability analyses for LI shell neurons, the omnibus LMM is included below:

Phase 3: LMM Omnibus Results for Low Intake, Shell Neurons				
Variables:	dF	N	F Value	P Value
Tone	**1**	16373	38.40	**<.0001**
Response	1	16373	.02	>.10
STNTGT	**2**	**16373**	**4.28**	**.014**
Titration	1	16373	2.00	>.10
Tone * Response	1	16373	.17	>.10
Tone * Titration	1	16373	.45	>.10
STNTGT * Titration	2	16373	6.16	.0021
STNTGT * Response	2	16373	.66	>.10
Response * Titration	1	16373	3.24	.07
Tone * STNTGT	2	16373	2.58	.076
STNTGT * Response * Titration	2	16373	.67	>.10
Tone * STNTGT * Response	2	16373	.21	>.10
Tone * Titration * STNTGT	**2**	16373	**17.88**	**<.0001**
Tone * Response * Titration	**1**	**16373**	**13.66**	**.0002**
Tone * Response * Titration * STNTGT	2	16373	3.18	>.10

The analysis of tone-evoked activity first analyzed Hits and Misses separately among only LI categorical variables (Titrate and NoTitrate, ST and NT and GT). All stability analysis results are indicated in Supplemental Table 3a2 and graphically represented in Figure 21a. Tone-evoked changes were present among many LI shell Neurons for different combinations of Titrate or NoTitrate during Hits and Misses: ST LI Miss NoTitrate slope= 0.178, 95% CI [-0.113, 0.468], intercept=0.030, p=.230 (not different vs. 0), ST LI Hit NoTitrate slope= 0.018, 95% CI [-

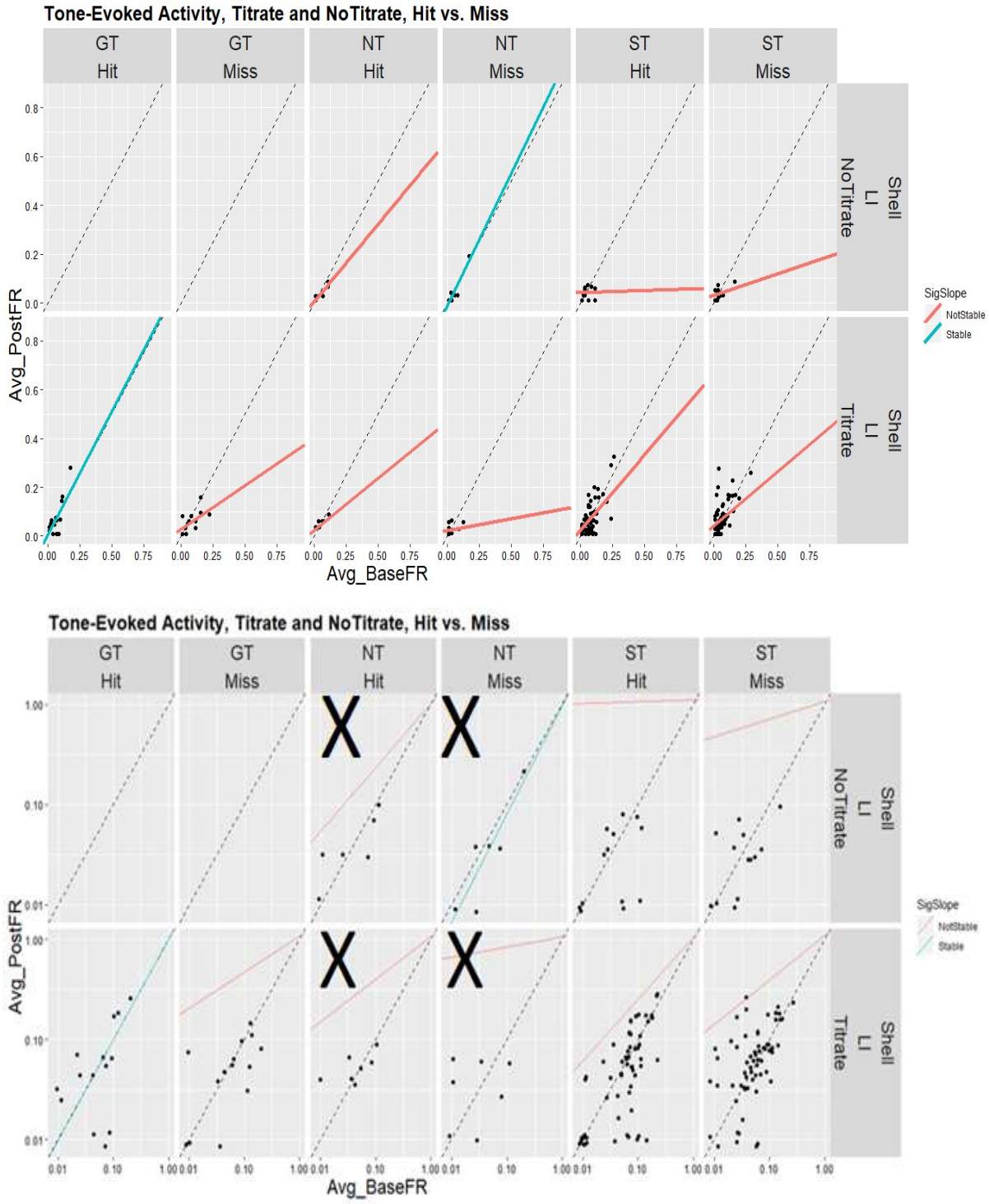

Figure 21a: tone evoked changes for shell, Low Intake, ST and NT and GT, Titrate and NoTitrate, with Hits and Misses separated. The x axis is the Mean Baseline FR and the y axis is the Mean Posttone FR for a particular Neuron from the specific subcategory of STNTGT, Titration, and Response. Color of lines indicate if the slopes (Baseline vs. Posttone) were stable (Blue, Baseline vs. Posttone no different) or not stable (Red,

Baseline vs. Posttone different, tone-evoked). The top figure represents raw FR scatterplots, and the bottom figure represents log scaled axes to enhance the location of individual neuron regressions. The “X” in the top left signals a significant difference in slopes between Hits vs. Misses, an open circle “O” in the top left signals a significant difference in Average Baseline FRs between Hits vs. Misses, and a merged x and open circle “⊗” indicates both (Hit vs. Miss slope differences, and Hit vs. Miss baseline differences). There were no LI GT NoTitrate neurons present in the study. As indicated above by colors: tone-evoked changes were present among many LI shell Neurons for different combinations of Titrate or NoTitrate during Hits and Misses: ST LI Miss NoTitrate, ST LI Hit NoTitrate, NT LI Hit NoTitrate, ST LI Miss Titrate, NT LI Miss Titrate, GT LI Miss Titrate, ST LI Hit Titrate, NT LI Hit Titrate. As indicated above by symbols: NT LI Titrate neurons showed differential tone processing on Hits vs. Misses, as did NT LI NoTitrate. There was no evidence of differential Hit vs. Miss slopes for any other category or any Baseline FR differences observed (see Supplemental Table 3a2)

0.276, .312], intercept= 0.043, p=.904 (not different vs. 0), NT LI Hit NoTitrate slope= 0.635, 95% CI [0.449, 0.821] , intercept= 0.006, ST LI Miss Titrate slope= 0.445, 95% CI [0.201 , 0.690], intercept= 0.042, NT LI Miss Titrate slope= 0.100, 95% CI [-0.178, 0.378] , intercept= 0.021, p=.482 (not different vs. 0), GT LI Miss Titrate slope= 0.357, 95% CI [-0.033, 0.748] , intercept=0.030, p=.073 (not different vs. 0), ST LI Hit Titrate slope= 0.617, 95% CI [0.237, 0.998] , intercept= 0.023, NT LI Hit Titrate slope= 0.425, 95% CI [0.142, 0.708], intercept= 0.026. Results revealed ST LI shell tone-evoked changes for both Hits and Misses among Titrate and NoTitrate groups, and the same results for NT LI Titrate and NoTitrate groups.

Slopes were then compared for any categorical variable that demonstrated tone-evoked changes on either Hits or Misses for shell LI neurons and it should be noted that adjusted p values could not be computed due to lack of GT LI NoTitrate neurons, and thus typical p values are reported. Results suggested only NT LI Titrate neurons showed differential tone processing on Hits vs. Misses, $Diff_{Slopes}$= 0.325, t(16356)=1.97, p=0.048 as did NT LI NoTitrate $Diff_{Slopes}$= -0.449, t(16356)=-3.67, p=0.0002. There was no evidence of differential Hit vs. Miss slopes for any other category; ST LI Titrate $Diff_{Slopes}$= .172, t(16356)=1.08, p>.10 and ST LI NoTitrate $Diff_{Slopes}$= -0.160, t(16356)=.165, p>.10 and GT LI Titrate $Diff_{Slopes}$= 0.650, t(16356)=1.73, p=.083 were all not significant.

We then tested mean baseline FR differences in Hit vs. Misses for every subcategory of Titration for LI shell neurons. For purposes of completeness the omnibus GLMM are included in the table below:

Phase 3: Baseline FR Comparisons, LMM Omnibus Results for LI Shell Neurons				
Variables:	dF	N	F Value	P Value
Response	1	16366	.18	>.10
STNTGT	2	16366	.22	>.10
Titration	1	16366	1.20	>.10
Response * Titration	1	16366	.29	>.10
STNTGT * Titration	**2**	**16366**	**4.66**	**.031**
Response * STNTGT	2	16366	1.67	>.10
Response * Titration * STNTGT	2	16366	.48	>.10

Thus, LI shell neurons exhibited tone-evoked changes for Hits and/or Misses in GT, NT, and ST Titrate and NoTitrate groups. Furthermore, only NT Titrate and NT NoTitrate groups differentially processed the tone on Hits and Misses and there was no influence of Baseline FR for any Response for each subcategory.

Low Intake Core Phase 3

For purposes of generating the post hoc stability analyses for only HI core groups the omnibus LMM are included below:

Phase 3: LMM Omnibus Results for Low Intake, Core Neurons				
Variables:	dF	N	F Value	P Value

Tone	**1**	**10110**	**59.71**	**<.0001**
Response	1	10110	.92	>.10
STNTGT	2	10110	.44	>.10
Titration	1	10110	.96	>.10
Tone * Response	1	10110	.15	>.10
Tone * Titration	1	10110	.08	>.10
STNTGT * Titration	2	10110	1.27	>.10
STNTGT * Response	2	10110	.88	>.10
Response * Titration	1	10110	5.28	.022
Tone * STNTGT	2	10110	1.08	>.10
STNTGT * Response * Titration	2	10110	.78	>.10
Tone * STNTGT * Response	**2**	**10110**	**5.70**	**.003**
Tone * Titration * STNTGT	2	10110	3.00	.084
Tone * Response * Titration	1	10110	1.59	>.10
Tone * Response * Titration * STNTGT	2	10110	.17	>.10

The analysis of tone-evoked activity first analyzed Hits and Misses separately among just LI core categorical variables (Titrate and NoTitrate, ST and NT and GT). All stability analysis results are indicated in Supplemental Table 3b2 and graphically represented in Figure 21b. Both Hits and Misses showed evidence of tone-evoked changes for only ST LI NoTitrate where ST Miss NoTitrate slope= 0.665, 95% CI [0.440, 0.891], intercept= 0.006 and ST Hit NoTitrate slope= 0.438, 95% CI [-0.118, 0.995],

intercept= 0.030, p=.123 (not different vs. 0). Tone-evoked changes also occurred in NT Miss NoTitrate slope= 1.405, 95% CI [1.047, 1.764], intercept= -0.032 and GT Miss Titrate slope= 0.510, 95% CI [0.414, 0.606], intercept= 0.022 and NT Hit

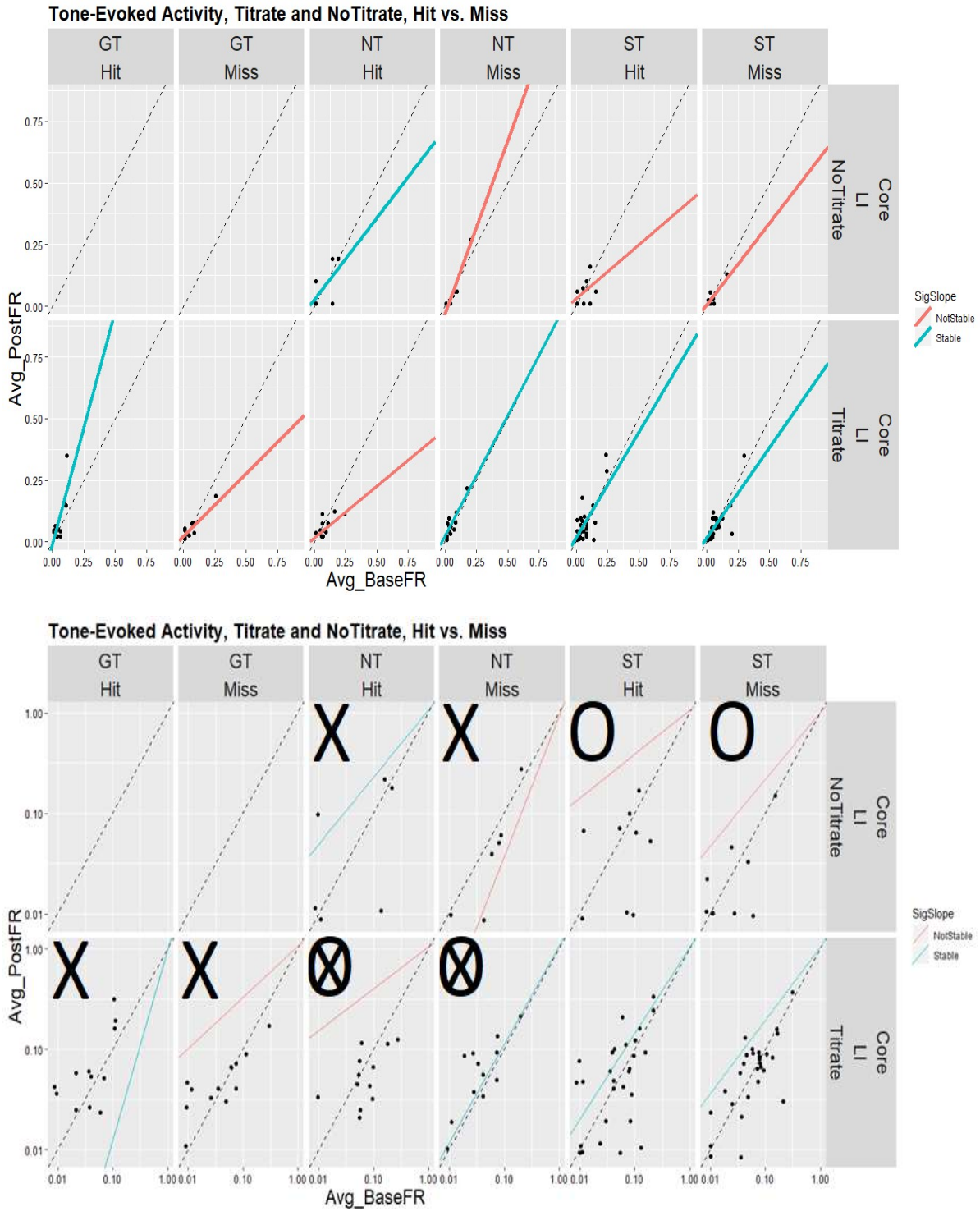

Figure 21b: tone evoked changes for core, Low Intake, ST and NT and GT, Titrate and NoTitrate, with Hits and Misses separated. The x axis is the Mean Baseline FR and the y axis is the Mean Posttone FR for a particular Neuron from the specific subcategory of STNTGT, Titration, and Response. Color of lines indicate if the slopes (Baseline vs. Posttone) were stable (Blue, Baseline vs. Posttone no different) or not stable (Red, Baseline vs. Posttone different, tone-evoked). The

top figure represents raw FR scatterplots, and the bottom figure represents log scaled axes to enhance the location of individual neuron regressions. The “X” in the top left signals a significant difference in slopes between Hits vs. Misses, an open circle “O” in the top left signals a significant difference in Average Baseline FRs between Hits vs. Misses, and a merged x and open circle “⊗” indicates both (Hit vs. Miss slope differences, and Hit vs. Miss baseline differences). There were no observed GT LI NoTitrate neurons in this study. As indicated above by colors: ST Miss NoTitrate, ST Hit NoTitrate, NT Miss NoTitrate, GT Miss Titrate, and NT Hit Titrate. As indicated above by symbols: tone-evoked differences on Hits vs. Misses were present for GT LI Titrate, NT LI Titrate, and NT LI NoTitrate. Baseline FR differences were observed among NT LI Titrate core neurons ($p<.0001$) and for ST LI NoTitrate core neurons ($p=.045$). Complete results are indicated in Supplemental Table 3b2.

Titrate slope= 0.418, 95% CI [0.195, 0.640], intercept= 0.018. ST Titrate neurons did not show Hit or Miss tone-evoked activity while all other groups resulted in at least one Response category with a tone-evoked change.

Slopes were then compared for any categorical variable that demonstrated tone-evoked changes on either Hits or Misses (i.e., every category except LI ST Titrate core neurons). It should be noted that adjusted p values could not be computed due to lack of GT LI NoTitrate neurons, so typical p values are reported here. Results suggested tone-evoked differences on Hits vs. Misses for GT LI Titrate $Diff_{Slopes}$= 1.386, t(10038)= 2.24, p=.025 and NT LI Titrate $Diff_{Slopes}$= -0.561, t(10038)= -2.70, p=.007, and NT LI NoTitrate $Diff_{Slopes}$= -0.742, t(10038)= -2.39, p=.017. There was no evidence of slope differences present for ST LI NoTitrate neurons $Diff_{Slopes}$= -0.227, t(10038)= -.92, p>.10.

We then tested mean baseline FR differences in Hit vs. Misses for every subcategory of LI core neurons. For purposes of completeness the omnibus GLMM and are included in the table below:

Phase 3: Baseline FR Comparisons, LMM Omnibus Results for LI Core Neurons				
Variables:	dF	N	F Value	P Value
Response	1	10048	.61	>.10
STNTGT	2	10048	.61	>.10
Titration	1	10048	1.20	>.10
Response * Titration	1	10048	0.00	>.10
STNTGT * Titration	2	10048	.96	>.10

Response * STNTGT	2	10048	1.31	>.10
Response * Titration * STNTGT	**2**	**10048**	**8.00**	**.005**

Post hoc analyses did reveal significant baseline FR differences between Hits vs. Misses for the subcategory of NT LI Titrate Core neurons ($p<.0001$) and for ST LI NoTitrate Core neurons ($p=.045$).

Therefore the differences in tone-evoked activity found in the slope comparisons of Hit vs. Miss was preserved for GT LI Titrate and NT NoTitrate. That is, no baseline FR differences were present that may have influenced comparisons of Hit vs. Miss slopes.

5.0 Discussion

SUD does not discriminate, and impacts individuals of all socioeconomic backgrounds (Grant *et al*., 2006). Furthermore, a troubling component of SUD is the influence of drug associated cues which act as a "trigger" for intense drug craving in humans (Ehrman *et al.,* 1992; Volkow *et al*., 2008) and animal models (Waters *et al*., 2014). Drug-cues act by reinvigorating previous drug associations within the mesolimbic dopamine system in areas such as the NAc shell (Ghitza *et al.,* 2003) and core (Hollander & Carelli 2005). In this 15 day cocaine SA study, our experiment utilized intermittent drug-availability signaled by a specific tone-cue S^D. The presentation of the cue at variable intervals enabled animals to choose to take drug ("Hit") or avoid opportunities to self-administer ("Miss") on each trial. We hypothesized the same NAc neuron would process the tone-cue S^D differentially during Hits (i.e., when the cue was thought to be highly salient and motivational because it actually triggered drug seeking) vs. when the cue evoked no response during Misses.

Researchers interested in predisposition to incentivize drug cues have implicated ST phenotype as a higher risk SUD group (Piazza *et al.*, 2000; Flagel *et al.,* 2007; Robinson & Flagel, 2009; Flagel *et al.*, 2010; Saunders, Yager & Robinson, 2013) because of ST predisposition to incentivize sucrose reward cues (Robinson & Berridge, 1993; Robinson & Flagel 2009; Flagel *et al.,* 2010). We tested the influence of ST, GT, and NT phenotype on overall drug consumption, behavioral discrimination of drug cues (the tone-cue S^D), and NAc core and shell processing of drug-related cues when the tone-cue S^D evoked a Hit vs. a Miss.

1. *We hypothesized no difference in overall drug consumption between ST NT and GT groups.*

Based on literature suggesting that drug intake does not differ across ST and GT phenotype (Tunstall & Kearns 2015; Bardo *et al.*, 2006) we hypothesized that all groups would self-administer the same quantity of cocaine across the entirety of the experiment. In support of this hypothesis, drug consumption (in mg/kg) did not differ across a continuum of pretest LP (Fig. 9) which indicated that the degree of "Sign-Tracking" did not influence the amount of cocaine consumed. In further support of this hypothesis, we found that ST, GT, and NT groups escalated drug intake similarly over the course of SA sessions (Fig. 10). This measure was particularly important because "escalation" is known as a key factor of addiction in animal models (Ahmed & Koob, 1998) and a major criterion of SUD in humans (National Institute on Drug Abuse; National Institutes of Health; U.S. Department of Health and Human Services. 2018). An additional "risk" factor in the severity of SUD is preference for high drug level (Wolffgramm & Hyene 1995) and we did find more high-intake ("HI") sessions among GT vs. ST, but no difference in HI sessions among GT vs. NT, nor NT vs. ST. However, the category of HI (or LI) was identified based on a median split of DL, and was designed to independently evaluate HI situations from LI (not to compare across HI/LI categories). The differences found between GT and ST may have been an artifact of the binary split of HI or LI for each given session and should not be considered the main criterion in evaluation of this hypothesis given the overall similarity of session drug intake (Fig. 9)

and escalation of intake (Fig. 10). Nonetheless, our findings indicate that the GT phenotype must be considered at least as much at risk for drug abuse as other groups based on sign tracking tendencies.

2. We hypothesized differences would appear across STNTGT for tone-discrimination.

The purpose of this study was to identify NAc processing of the tone-cue S^D, and with literature implicating ST phenotype as highly reward cue sensitive we hypothesized ST would behaviorally discriminate the tone-cue S^D more effectively than GT or NT. However, we found no evidence of "better" tone-discrimination during Predrug trials, as all three groups acquired the tone and task identically across sessions (Fig. 8), which confirmed ST did not *learn* or perform the task faster than other groups.

ST also did not demonstrate a "better" ability to discriminate the tone during the Maintenance phase of SA relative to other groups. This was true for all LI GT, NT, and ST groups which did not differ in any aspect of Maintenance Uncued Responding (Fig. 12a-d) while Uncued Responding was equally high among GT HI and ST HI (Fig. 12a-d). The only phenotype differences observed were between GT HI vs. NT HI (Fig. 12a, d). Interestingly, elevated Uncued Responding was present among HI vs. LI in general (Fig. 12a-d) and video analysis suggested HI subjects spent almost the entirety of the session in/near the operant corner (Fig. 17). These results suggest ST were just as likely as GT and NT to lack or abandon tone discrimination when drug level was elevated during Maintenance.

This elevated Uncued Responding among HI subjects in general could have been due to the unpredictable timing of tone presentations, which set the occasions of drug availability. Specifically, the Maintenance phase utilized a pseudo-randomized variable intertrial interval of 1-6 minutes, and animals needed to choose within 30 seconds to self-administer drug (Hit) or avoid drug opportunities (Miss) upon the onset of the tone-cue S^D. In this way, we expected animals would "titrate" DL and Miss when DL was high, and Hit when DL was low (Pickens, Thompson & Yokel 1972), consistent with theories of negative reinforcement in which animals take drug to delay inevitable drug withdrawal (Zimmer *et al.*, 2013; Barker *et al.*, 2014). We anticipated that most animals would also learn to self-administer drug in relation to the randomized tone-onset.

In this way we were able to explore an additional aspect of tone-discrimination by measuring the difference between DL on Hits vs. Misses over the course of the session and hypothesized "Titration" would be most apparent among ST. Unexpectedly ST subjects were no "better" than other groups at Titration. In fact, both GT and ST were able to Titrate when average session DL was low but exhibited greater incidence of NoTitrate at high drug levels. This was revealed by a regression of raw DLDifference scores (Fig. 11), and by a comparison of the raw number of "NoTitrate" sessions between HI and LI of the same phenotype (Fig. 13). Further analyses utilized the factor "Titration" and we evaluated Titrate situations (High Miss DL) independently from NoTitrate (High Hit DL) separately among HI and LI groups because "NoTitrate" was considered a measure of high risk and uncontrolled drug intake reflected in humans

suffering from SUD (National Institute on Drug Abuse; National Institutes of Health; U.S. Department of Health and Human Services. 2018).

Analysis of Maintenance Uncued vs. Cued Response Rate revealed significantly higher Cued Response Rate for almost every subgroup of HI and LI, Titrate and NoTitrate, ST/GT/NT (except for NT HI NoTitrate) (Fig. 14). This finding appears to match the Predrug measure of tone discrimination. However, during Maintenance, the high Cued Response rate for GT and ST HI NoTitrate groups may have been due to continual responding, irrespective of the tone-cue S^D. This was evidenced by the finding that both HI GT NoTitrate and HI ST NoTitrate (Fig. 13) emitted more than half of all Hits within 1 second after onset of the tone-cue S^D (ST NoTitrate=55%, and GT NoTitrate=51%). These same groups also exhibited the same high rate of ongoing nosepokes during the one second immediately *before* tone onset on Hits, providing further evidence of continual responding. We then compared Maintenance Uncued Response Rate between Titration groups of the same Intake and Phenotype. We found significantly higher Uncued Response Rate among GT HI NoTitrate compared to GT HI Titrate and this result, combined with high Cued Response Rate and continual responding among GT HI NoTitrate, suggested GT HI NoTitrate had high Response Rate in general with no specific evidence of "tone-discrimination." Conversely, GT HI Titrate *did* discriminate the tone based on their relatively low Uncued Response Rate and High Cued Response rate. We did not identify differences in Uncued Response Rate between ST HI NoTitrate vs. ST HI Titrate (Fig. 14) and inspection of Figure 14 suggested that both ST HI Titrate and

NoTitrate groups were equally high in Uncued Response Rate, providing evidence of poor tone-discrimination across all ST HI groups regardless of Titration category.

All these results suggested our hypothesis that ST would be universally "better" at tone-discrimination was not supported. For example, we determined that ST were no different from any other group regarding tone discrimination during Predrug (Fig. 8) or Maintenance (Fig. 12). Furthermore, during Maintenance ST HI were just as likely as GT HI to lack tone-discrimination (Fig. 12), with both groups unable to Titrate at high drug levels (Fig. 11, Fig. 13). These same HI NoTitrate groups demonstrated continual responding immediately before and after onset of the tone-cue S^D (Fig. 13). We also observed no difference in Uncued vs. Cued Response Rate for NT HI NoTitrate (Fig. 14), higher Uncued Response Rate for GT HI NoTitrate relative to GT Titrate (Fig. 14), and equally high levels of Uncued Response Rate between ST Titrate and ST NoTitrate (Fig. 14). Therefore, GT HI NoTitrate, NT HI NoTitrate, and all ST HI (NoTitrate and Titrate) did not exhibit evidence of discriminating the tone during Maintenance. These high drug intake groups that were not titrating may be more sensitive to, or more motivated by drug levels that, even though high, are below a currently desired level. Thus, because "Not Titrating" was defined as hitting when DL was high AND missing when DL was low, the findings suggest that situations of intermittent, unpredictable drug availability may create risk of excessive intake/abuse for all subjects across the ST/GT spectrum.

3. *We hypothesized that Core and Shell neurons from ST (specifically HI) would process the tone on both Hits and Misses due to historical precedence of ST being highly "cue-sensitive".*

It is important to note that all neural comparisons were made for the same neuron at different timepoints (i.e., Baseline vs. Posttone, and Hits vs. Misses). Also, analyses were separated into multiple phases in order to inspect how the onset of the tone-cue S^D changed FR from Baseline to Posttone for core or shell neurons of specific subgroups.

Our first analysis was designed to identify tone-evoked changes in general while ignoring different Response and Titration categories. Contrary to expectations, we did not identify tone evoked changes in shell (Fig. 18a) or core (Fig. 18b) in the ST group, either for HI or LI. Instead, we identified tone-evoked changes in GT HI and LI shell neurons (Fig. 18a) and GT HI core neurons (Fig. 18b). However, our major scientific interest involved comparisons of how the same NAc neuron changed upon the onset of the tone-cue S^D for Hits compared to Misses. The reason for this direct comparison was that Hits were assumed to be associated with a higher motivation and salience to the tone-cue S^D, while Misses were assumed to have lower salience to the tone-cue S^D. Firing on Hits but not Misses might provide physiological evidence of the long-held concept that the NAc is anatomically positioned to gate limbic signals to premotor regions in preparation for action. We anticipated this would be reflected in tone-evoked changes on Hits compared to Misses because of previous findings suggesting strong cue-evoked changes in NAc shell FR during the first extinction trials (Ghitza *et al.,* 2003) and in core neurons during drug cue presentation (Hollander & Carelli 2005). We also hypothesized that this trend would be particularly localized among ST HI neurons because of previously mentioned cue-sensitivity found in other studies.

Before we made any direct comparisons between Hits vs. Misses we first confirmed that there were no differences in locomotor velocity between these two response types (Fig. 15). This first step was critical because NAc neurons are influenced by motor behavior (Coffey *et al.,* 2015). Secondly, we found that high drug level was associated with lower velocity in general on both Hits and Misses (Fig. 16). This is consistent with a reduction in locomotion and rearing as animals transition to focused stereotypy when stimulant drugs are onboard at high levels (Lyon & Robbins, 1975; Segal, 1976).

Given these controls for velocity differences, any tone-evoked processing differences on Hits vs. Misses were free of motoric interference. For shell neurons, we identified tone-evoked changes for at least one response category (either Hits or Misses) for every HI and LI group (Fig. 19a). However, contrary to expectations, HI shell neurons were associated with tone-evoked changes only on Misses but not Hits (Fig. 19a). Furthermore, only GT HI shell neurons showed significantly different slopes on Hits (positive change) vs. Misses (negative change). This was also contrary to expectations that ST HI neurons would exhibit the strongest difference in tone-evoked changes between Hits and Misses.

For core neurons, we did not identify any differences in tone-evoked activity on either Hits or Misses for ST HI or LI (Fig. 19b). Instead, tone-evoked changes were present in both GT HI Hits and Misses but the processing was not different between the two types of response (Fig. 19b). NT HI Misses (but not Hits) showed weak tone-evoked changes (Fig. 19b) and the only evidence of differential tone-processing on Hits vs. Misses in core neurons for this phase was for NT LI. Therefore, there was tone-evoked

activity for certain combinations of Response, Intake, and STNTGT. However, our hypothesis that ST would show strong tone-processing differences on Hits vs. Misses was not supported for either HI or LI. In fact, ST HI and LI did not show any tone-evoked activity for core neurons. Unexpectedly, GT HI did show tone-evoked activity on Hits and Misses, but there were no differences across Hits and Misses.

The third and final phase of the analysis was to address the influence of Titration on tone-evoked processing for core and shell neurons between Hits and Misses for HI and LI groups. The inclusion of Titration as a factor was critical because high cocaine level can suppress FR among NAc neurons (Peoples & West, 1996; Nicola & Deadwyler, 2000) and we were also interested in how HI (i.e., potential high risk) groups processed the tone on days when DL could not be "controlled" (i.e., NoTitrate) where DL was elevated on Hits but low on Misses, and thus was contradictory to expected patterns of self-administration.

The results of this analysis suggested tone-evoked changes on Misses were present among all HI shell phenotypes, regardless of Titration. However, differences in Hit vs. Miss tone-evoked changes were present only among GT HI Titrate, and NT HI Titrate groups (Fig. 20a). These findings were contrary to expectations regarding ST HI neurons, in that we found no differences in tone-evoked NAc firing between Hit vs. Miss for ST HI Titrate or ST HI NoTitrate groups. While these results did not support our prediction of strong tone-evoked processing in the NAc of ST, we instead uncovered a different trend: HI groups that discriminated the tone behaviorally (i.e., GT HI Titrate, NT HI Titrate) showed significantly different tone-processing on Hits (no change) vs. Misses (strong

change). That is, a key finding was that when HI subjects did not discriminate the tone behaviorally (i.e., all ST HI groups, GT HI NoTitrate, NT HI NoTitrate) their NAc shell neurons did not reflect differences between Hits (no change) vs. Misses (weak change).

For HI core neurons, we identified tone-evoked changes almost universally on Hits and Misses for HI groups (except for NT HI Titrate Hits) but no differences existed between Hits vs. Misses (Figure 20b). Therefore, our hypothesis was marginally supported among ST HI Core neurons in that tone-evoked changes were present for both Hits and Misses in Titrate and NoTitrate groups. However, there were no differences in tone-evoked changes between Hits and Misses for either ST HI group. Furthermore, the lack of tone-evoked differences between Hits and Misses in core was the same for every group. Thus our hypothesis that ST HI NAc tone-processing would be "stronger" was not supported.

For LI shell neurons, tone-evoked activity was also present for Misses in all categories (except NT LI NoTitrate) and for Hits in all categories (except GT LI Titrate). However, tone-evoked activity was different between Hits and Misses only for NT LI groups (Fig. 21a). For LI Core neurons, tone-evoked changes were present in either Hits or Misses for every subcategory of STNTGT and Titration (except ST LI Titrate) and differences in tone-evoked activity between Hits and Misses were present among GT LI Titrate, NT LI Titrate, and NT LI NoTitrate (Fig 21b). That is, for groups other than ST, during sessions of Low Intake, core neurons and to a lesser degree shell neurons processed the tone differently on Hits vs Misses. Taken together, tone-evoked

processing was not “stronger” among LI core or shell neurons in the ST group under any condition, and thus our hypothesis was not supported.

4. Summary

Overall these findings demonstrate that when HI animals were on task and processed the tone behaviorally (i.e., low responding during tone-off: GT HI Titrate and NT HI Titrate), their NAc shell neurons reflected the underlying tone-discrimination. In addition, while our hypothesis of stronger tone-evoked changes on Hits than on Misses was not supported, we instead observed the opposite: these same shell HI neurons showed significantly stronger tone-evoked changes on Misses compared to Hits. This difference in tone-processing, although unexpected, may be important nonetheless, *because* it is a difference. For example, stronger changes on Misses could feed forward to influence responding. It could have acted as a “stop” signal which would contribute to the ability of these groups to behaviorally discriminate the tone and titrate DL.

Further, we found that when HI animals were not on task, and did not process the tone behaviorally (i.e., continual responding regardless of tone-on/off: GT HI NoTitrate, NT HI NoTitrate, both ST HI groups), NAc shell neurons showed relatively weak tone-evoked changes on Misses which were not different from Hits. It is possible that the “stop signal” was ineffective for these animals, which may have been reflected in the observed high Hit DL relative to Miss DL (i.e., NoTitrate). However, this NAc shell firing pattern also appeared in ST HI Titrate neurons. Thus one alternative explanation is that the opportunity to self-administer (i.e., the onset of the tone-cue S^D) was consistently

salient in all of these subgroups (i.e., ST HI Titrate; all HI NoTitrate). This could would explain the continuous responding identified among these groups before the onset of the tone-cue S^D. Subjects may have responded in the corner as much as possible to minimize the risk of a "Miss", because the consequence of a Miss would be a random 1-6 minute interval in which drug would not be available. This is particularly relevant to the ST HI Titrate group, which self-administered drug in an expected pattern but still lacked the tone-evoked changes observed in GT HI and NT HI Titrate groups.

Overall, we identified tone-evoked differences on Hits vs. Misses in neurons recorded from animals considered to be "not severe" cases of SUD, even when animals self-administered large quantities of drug, as in GT HI Titrate, NT HI Titrate (assuming that Titrating reflects control over intake, compared to loss of control when Not Titrating). We identified a different NAc firing pattern among the "worst case" SUD models (i.e., all HI NoTitrate and all ST HI subjects) where the onset of the tone-cue S^D resulted in similar FR patterns on both Hits and Misses. These results suggest that in these "worst cases" the tone may have always been motivational or salient, based on how NAc shell neurons processed the tone-cue S^D similarly on Hits and Misses. This NAc shell pattern would reflect the most difficult aspects of SUD to treat in humans, where cues associated with drug use can spark craving and trigger relapse even after long periods of abstinence (Ehrman *et al.,* 1992; Volkow *et al*., 2008). For example, among humans in SUD recovery such external cues may not always result in a "Hit" response, but their internal processing of cues would be similar enough to promote strong urges or craving even when motivation to take drug is low (i.e., when not in drug withdrawal).

Reports have posited that ST are prone to cue-induced relapse because of predisposition to develop salience to reward cues (Piazza *et al.*, 2000; Flagel *et al.,* 2007; Robinson & Flagel, 2009; Flagel *et al.*, 2010; Saunders, Yager & Robinson, 2013). The lack of tone-evoked differences on Hits vs. Misses observed for all ST HI groups could be a result of the tone-cue S^D always being "salient" and motivational. For instance, when the outcome was a "Miss", ST HI NAc shell neurons exhibited weak tone-processing in general, and tone-processing was similar to a "Hit". The "stop signal" observed in the shell of other HI groups was absent. This could imply that ST may be predisposed to develop cue-induced relapse, consistent with 30 years of literature suggesting just that. Even though ST HI may not have "wanted" drug (on Misses), their neurons reflected similar firing patterns when subjects did "want" the drug (on Hits) and ST may be prone to develop cue induced relapse. Nonetheless, our findings demonstrate that other groups (GT and NT) are in no way "immune" to this phenomenon given the similarities identified among HI NoTitrate groups. Therefore, a "spectrum" of ST, NT, and GT groups must always be considered when making any claims regarding phenotype influences on substance abuse.

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7.0 Appendix

Region	Label	Slope	Intercept	Slope Std Error	DF	Pval: Slope_vs._0	95% Conf. lower	95% Conf. upper	Tone evoked?
Shell	Slope1:STGT=ST, Intake=LI	0.9125	0.006818	0.07277	27637	<.0001	0.7699	1.0552	No
Shell	Slope2:STGT=NT, Intake=LI	0.6454	0.01417	0.2315	27637	0.0053	0.1916	1.0992	No
Shell	Slope3:STGT=GT, Intake=LI	0.5436	0.01446	0.1116	27637	<.0001	0.3249	0.7623	Yes
Shell	Slope4:STGT=ST, Intake=HI	0.8338	0.01737	0.1798	27637	<.0001	0.4814	1.1862	No
Shell	Slope5:STGT=NT, Intake=HI	0.6591	0.01914	0.2117	27637	0.0018	0.2442	1.074	No
Shell	Slope6:STGT=GT, Intake=HI	1.4895	-0.0294	0.1642	27637	<.0001	1.1676	1.8114	Yes
Core	Slope1:STGT=ST, Intake=LI	1.1076	-0.0044	0.122	17940	<.0001	0.8685	1.3467	No
Core	Slope2:STGT=NT, Intake=LI	0.8575	0.01012	0.1113	17940	<.0001	0.6394	1.0756	No
Core	Slope3:STGT=GT, Intake=LI	1.4167	-0.01271	0.3154	17940	<.0001	0.7985	2.035	No
Core	Slope4:STGT=ST ,Intake=HI	1.0783	0.000939	0.2661	17940	<.0001	0.5568	1.5998	No
Core	Slope5:STGT=NT, Intake=HI	0.8515	0.01004	0.1319	17940	<.0001	0.5929	1.1101	No
Core	Slope6:STGT=GT, Intake=HI	0.2612	0.03675	0.8257	17940	0.7518	-1.3573	1.8796	Yes

Supplemental Table 1: HM Collapsed stability analysis results for Core and Shell, High Intake and Low Intake, ST and NT and GT, Baseline vs. Posttone: the Pval for slope vs. 0 represents the significance level for complete lack of stability (and evidence of tone-evoked changes Baseline vs. Posttone). If 1.0 did not fall within 95% of the lower and upper confidence values (the far right columns) then the neuron was also considered to lack stability (and was also considered evidence of tone evoked change). The furthest right column is a summary of this data.

Reg	Label	Slope	Int.	Slope Std Error	DF	Pval V 0	95% Conf. lower	95% Conf. upper	Tone Ev?	H v M Cont. Estimate	Pr>|t|	Adj P	Sig Hit vs. M?
Shell	Slope1: STGT=ST, Resp=Miss, Intake=LI	0.4657	0.03592	0.1216	27622	0.0001	0.2273	0.704	Yes	0.1722	0.2094	0.5058	No
Shell	Slope2: STGT=NT, Resp=Miss, Intake=LI	0.6954	0.00359	0.3012	27622	0.0209	0.1051	1.2857	No	-0.1571	0.5687	0.814	No
Shell	Slope3: STGT=GT, Resp=Miss, Intake=LI	0.2898	0.03225	0.1816	27622	0.1106	-0.06622	0.6457	Yes	0.6061	0.1057	0.3603	No
Shell	Slope4: STGT=ST, Resp=Hit, Intake=LI	0.6378	0.01826	0.1903	27622	0.0008	0.2647	1.0109	No	0.1722	0.2094	0.5058	No
Shell	Slope5: STGT=NT, Resp=Hit, Intake=LI	0.5383	0.01762	0.1554	27622	0.0005	0.2337	0.8429	Yes	-0.1571	0.5687	0.814	No
Shell	Slope6: STGT=GT, Resp=Hit, Intake=LI	0.8959	0.01083	0.3422	27622	0.0088	0.2253	1.5665	No	0.6061	0.1057	0.3603	No
Shell	Slope7: STGT=ST, Resp=Miss, Intake=HI	0.5214	0.02148	0.1069	27622	<.0001	0.3119	0.7308	Yes	0.1621	0.5845	0.814	No
Shell	Slope8: STGT=NT, Resp=Miss, Intake=HI	0.205	0.03027	0.2284	27622	0.3694	-0.2426	0.6526	Yes	0.5607	0.0329	0.1542	No
Shell	Slope9: STGT=GT, Resp=Miss, Intake=HI	0.7354	0.03943	0.09534	27622	<.0001	0.5486	0.9223	Yes	0.5557	0.0023	0.0137	Yes
Shell	Slope10: STGT=ST, Resp=Hit, Intake=HI	0.6834	0.0317	0.2881	27622	0.0177	0.1186	1.2482	No	0.1621	0.5845	0.814	No
Shell	Slope11: STGT=NT, Resp=Hit, Intake=HI	0.7656	0.02069	0.1618	27622	<.0001	0.4486	1.0827	No	0.5607	0.0329	0.1542	No
Shell	Slope12: STGT=GT, Resp=Hit, Intake=HI	1.2911	-0.01521	0.1929	27622	<.0001	0.913	1.6692	No	0.5557	0.0023	0.0137	Yes

Supplemental Table 2a: Shell stability analysis results for Core and Shell, High Intake and Low Intake, ST and NT and GT, Hit and Miss, Baseline vs. Posttone: the Pval for slope vs. 0 represents the significance level for complete lack of stability (and evidence of tone-evoked changes Baseline vs. Posttone). If 1.0 did not fall within 95% of the lower and upper confidence values then the neuron was also considered to lack stability (and was also considered evidence of tone evoked change). The furthest right column is a comparison of Hit and Miss slopes summary of this data.

Reg	Label	Slope	Int.	Slope Std Error	DF	Pval_ Slope _vs._ 0	95% Conf. lower	95% Conf. upper	Tone Evoked?	H v M Cont. Estimate	Pr>|t|	Adj P	Sig Hit vs. M?
Core	Slope1: STGT=ST, Resp=Miss, Intake=LI	0.7418	0.0154	0.197	17925	0.0002	0.3556	1.128	no	0.1579	0.2776	0.7277	no
Core	Slope2: STGT=NT, Resp=Miss, Intake=LI	1.0877	0.009844	0.1071	17925	<.0001	0.8776	1.2977	no	-0.6755	<.0001	0.0004	yes
Core	Slope3: STGT=GT, Resp=Miss, Intake=LI	0.4844	0.02346	0.05755	17925	<.0001	0.3716	0.5972	yes	1.3678	0.0242	0.1154	no
Core	Slope4: STGT=ST, Resp=Hit, Intake=LI	0.8997	0.009817	0.2223	17925	<.0001	0.4641	1.3354	no	0.1579	0.2776	0.7277	no
Core	Slope5: STGT=NT, Resp=Hit, Intake=LI	0.4121	0.02034	0.1437	17925	0.0041	0.1305	0.6938	yes	-0.6755	<.0001	0.0004	yes
Core	Slope6: STGT=GT, Resp=Hit, Intake=LI	1.8522	-0.00537	0.6417	17925	0.0039	0.5945	3.1099	no	1.3678	0.0242	0.1154	no
Core	Slope7: STGT=ST, Resp=Miss, Intake=HI	0.9313	0.00803	0.2861	17925	0.0011	0.3706	1.492	no	-0.02324	0.9154	0.9154	no
Core	Slope8: STGT=NT, Resp=Miss, Intake=HI	0.858	0.0213	0.05974	17925	<.0001	0.7409	0.9751	yes	-0.04322	0.7069	0.9141	no
Core	Slope9: STGT=GT, Resp=Miss, Intake=HI	0.05617	0.0813	0.559	17925	0.92	-1.0395	1.1518	no	0.3783	0.3144	0.7277	no
Core	Slope10: STGT=ST, Resp=Hit, Intake=HI	0.9081	0.01502	0.1794	17925	<.0001	0.5566	1.2597	no	-0.02324	0.9154	0.9154	no
Core	Slope11: STGT=NT, Resp=Hit, Intake=HI	0.8148	0.01009	0.1566	17925	<.0001	0.5078	1.1217	no	-0.04322	0.7069	0.9141	no
Core	Slope12: STGT=GT, Resp=Hit, Intake=HI	0.4344	0.02614	0.4747	17925	0.3601	-0.4961	1.3649	yes	0.3783	0.3144	0.7277	no

Supplemental Table 2b: Core stability analysis results for Core, High Intake and Low Intake, ST and NT and GT, Hit and Miss, Baseline vs. Posttone: the Pval for slope vs. 0 represents the significance level for complete lack of stability (and evidence of tone-evoked changes Baseline vs. Posttone). If 1.0 did not fall within 95% of the lower and upper confidence values then the neuron was also considered to lack stability (and was also considered evidence of tone evoked change). The furthest right column is a comparison of Hit and Miss slopes summary of this data.

Inta	Titrat.	HM	Reg.	Label	Slope	Int.	Slope Std Error	DF	Pval Slope vs. 0	95% Conf. lower	95% Conf. upper	Tone Ev?	H v M Cont. Est.	P r>|t|	Adj P	Sig H v M
HI	No Titrate	Miss	Shell	Slope1: STGT=ST, Resp=Miss, N_T=No Titrate	0.4944	0.01523	0.1102	11055	<.0001	0.2783	0.7104	yes	0.1195	0.6518	0.88	no
HI	No Titrate	Miss	Shell	Slope2: STGT=NT, Resp=Miss, N_T=No Titrate	0.8049	0.01761	0.4965	11055	0.105	-0.1683	1.7782	yes	0.6717	0.2234	0.53	no
HI	No Titrate	Miss	Shell	Slope3: STGT=GT, Resp=Miss, N_T=No Titrate	0.4206	0.08596	0.3011	11055	0.1625	-0.1696	1.0107	yes	-0.0719	0.8823	0.88	no
HI	No Titrate	Hit	Shell	Slope4: STGT=ST, Resp=Hit, N_T=No Titrate	0.6139	0.0261	0.248	11055	0.0133	0.1278	1.0999	no	0.1195	0.6518	0.88	no
HI	No Titrate	Hit	Shell	Slope5: STGT=NT, Resp=Hit, N_T=No Titrate	1.4767	0.02057	0.7439	11055	0.0472	0.01859	2.9347	no	0.6717	0.2234	0.53	no
HI	No Titrate	Hit	Shell	Slope6: STGT=GT, Resp=Hit, N_T=No Titrate	0.3487	0.03438	0.2825	11055	0.2171	-0.205	0.9024	yes	-0.0719	0.8823	0.88	no
HI	Titrate	Miss	Shell	Slope7: STGT=ST, Resp=Miss, N_T=Titrate	-0.2735	0.06749	0.1868	11055	0.1432	-0.6396	0.0927	yes	1.4257	0.0383	0.14	no
HI	Titrate	Miss	Shell	Slope8: STGT=NT, Resp=Miss, N_T=Titrate	-0.06471	0.03821	0.1416	11055	0.6477	-0.3423	0.2128	yes	0.8039	<.0001	0.0002	yes
HI	Titrate	Miss	Shell	Slope9: STGT=GT, Resp=Miss, N_T=Titrate	0.8429	0.01243	0.07613	11055	<.0001	0.6937	0.9922	yes	0.4857	0.0066	0.03	yes
HI	Titrate	Hit	Shell	Slope10: STGT=ST, Resp=Hit, N_T=Titrate	1.1523	-0.0039	0.531	11055	0.03	0.1113	2.1932	no	1.4257	0.0383	0.14	no
HI	Titrate	Hit	Shell	Slope11: STGT=NT, Resp=Hit, N_T=Titrate	0.7392	0.01495	0.1889	11055	<.0001	0.3689	1.1094	no	0.8039	<.0001	0.0002	yes
HI	Titrate	Hit	Shell	Slope12: STGT=GT, Resp=Hit, N_T=Titrate	1.3287	-0.00292	0.2015	11055	<.0001	0.9337	1.7236	no	0.4857	0.0066	0.03	yes

Supplemental Table 3a1: Shell stability analysis results for High Intake Shell, ST and NT and GT, Titrate and NoTitrate, Hit and Miss, Baseline vs. Posttone: the Pval for slope vs. 0 represents the significance level for complete lack of stability (and evidence of tone-evoked changes Baseline vs. Posttone). If 1.0 did not fall within 95% of the lower and upper confidence values then the neuron was also considered to lack stability (and was also considered evidence of tone evoked change). The furthest right column is a comparison of Hit and Miss slopes summary of this data.

Int	Titration	HM	Region	Label	Slope	Intercept	Slope Std Error	DF	Pval Slope vs. 0	95% Conf. lower	95% Conf. upper	Tone Evoked?	Estimate	Pr>|t|	Adj P	Sig H v M
LI	NoTitrate	Miss	Shell	Slope1: STGT=ST, Resp=Miss, N_T=NoTitrate	0.1779	0.030288	0.1481	16356	0.2298	-0.1125	0.4682	Yes	-0.1599	0.3312	.	no
LI	NoTitrate	Miss	Shell	Slope2: STGT=NT, Resp=Miss, N_T=NoTitrate	1.0837	-0.010604	0.1433	16356	<.0001	0.8029	1.3645	No	-0.4488	0.0002	.	yes
LI	NoTitrate	Miss	Shell	Slope3: STGT=GT, Resp=Miss, N_T=NoTitrate	Non-est	Non-est	Non-est	Non-est	Non-est	Non-est	Non-est					
LI	NoTitrate	Hit	Shell	Slope4: STGT=ST, Resp=Hit, N_T=NoTitrate	0.01801	0.043433	0.1498	16356	0.9043	-0.2757	0.3117	Yes	-0.1599	0.3312	.	no
LI	NoTitrate	Hit	Shell	Slope5: STGT=NT, Resp=Hit, N_T=NoTitrate	0.6349	0.005866	0.09507	16356	<.0001	0.4486	0.8213	Yes	-0.4488	0.0002	.	yes
LI	NoTitrate	Hit	Shell	Slope6: STGT=GT, Resp=Hit, N_T=NoTitrate	Non-est	Non-est	Non-est	Non-est	Non-est	Non-est	Non-est					
LI	Titrate	Miss	Shell	Slope7: STGT=ST, Resp=Miss, N_T=Titrate	0.4452	0.042111	0.1247	16356	0.0004	0.2009	0.6896	yes	0.1721	0.2795	.	no
LI	Titrate	Miss	Shell	Slope8: STGT=NT, Resp=Miss, N_T=Titrate	0.09978	0.021099	0.1418	16356	0.4815	-0.1781	0.3776	Yes	0.3251	0.0484	.	yes
LI	Titrate	Miss	Shell	Slope9: STGT=GT, Resp=Miss, N_T=Titrate	0.3572	0.029664	0.1992	16356	0.073	-0.03324	0.7477	Yes	0.6504	0.0832	.	no
LI	Titrate	Hit	Shell	Slope10: STGT=ST, Resp=Hit, N_T=Titrate	0.6173	0.023327	0.1941	16356	0.0015	0.2368	0.9978	Yes	0.1721	0.2795	.	no
LI	Titrate	Hit	Shell	Slope11: STGT=NT, Resp=Hit, N_T=Titrate	0.4249	0.025644	0.1444	16356	0.0033	0.1419	0.7078	Yes	0.3251	0.0484	.	yes
LI	Titrate	Hit	Shell	Slope12: STGT=GT, Resp=Hit, N_T=Titrate	1.0077	0.007257	0.365	16356	0.0058	0.2923	1.7233	No	0.6504	0.0832	.	no

Supplemental Table 3a2: Shell stability analysis results for Low Intake Shell, ST and NT and GT, Titrate and NoTitrate, Hit and Miss, Baseline vs. Posttone: the Pval for slope vs. 0 represents the significance level for complete lack of stability (and evidence of tone-evoked changes Baseline vs. Posttone). If 1.0 did not fall within 95% of the lower and upper confidence values then the neuron was also considered to lack stability (and was also considered evidence of tone evoked change). The furthest right column is a comparison of Hit and Miss slopes summary of this data.

Intake	Titration	HM	Region	Label	Slope	Intercept	Slope Std Error	DF	Pval_Slope v 0	95% Conf. lower	95% Conf. upper	Tone Evoked?	Estimate	Pr>|t|	Adj P	Sig H v M
HI	NoTitrate	Miss	Core	Slope1: STGT=ST, Resp=Miss, N_T=NoTitrate	0.6005	0.013618	0.3238	7853	0.0637	-0.03423	1.2353	yes	-0.0474	0.8599	0.9972	no
HI	NoTitrate	Miss	Core	Slope2: STGT=NT, Resp=Miss, N_T=NoTitrate	0.7984	0.035088	0.1	7853	<.0001	0.6023	0.9944	yes	0.030113	0.8037	0.9972	no
HI	NoTitrate	Miss	Core	Slope3: STGT=GT, Resp=Miss, N_T=NoTitrate	0.3999	0.104	0.9939	7853	0.6874	-1.5483	2.2382	yes	-0.2487	0.7702	0.9972	no
HI	NoTitrate	Hit	Core	Slope4: STGT=ST, Resp=Hit, N_T=NoTitrate	0.5531	0.016455	0.1203	7853	<.0001	0.3172	0.789	yes	-0.0474	0.8599	0.9972	no
HI	NoTitrate	Hit	Core	Slope5: STGT=NT, Resp=Hit, N_T=NoTitrate	0.8285	-0.00405	0.07769	7853	<.0001	0.6762	0.9808	yes	0.030113	0.8037	0.9972	no
HI	NoTitrate	Hit	Core	Slope6: STGT=GT, Resp=Hit, N_T=NoTitrate	0.1512	0.039822	0.2929	7853	0.6057	-0.423	0.7254	yes	-0.2487	0.7702	0.9972	no
HI	Titrate	Miss	Core	Slope7: STGT=ST, Resp=Miss, N_T=Titrate	2.2197	-0.04595	0.4658	7853	<.0001	1.3065	3.1328	yes	-1.0738	0.0137	0.0797	no
HI	Titrate	Miss	Core	Slope8: STGT=NT, Resp=Miss, N_T=Titrate	0.8298	0.022213	0.0728	7853	<.0001	0.6871	0.9725	yes	0.016117	0.9262	0.9972	no
HI	Titrate	Miss	Core	Slope9: STGT=GT, Resp=Miss, N_T=Titrate	-0.1165	0.046866	0.1134	7853	0.3043	-0.3388	0.1058	yes	0.4164	0.3111	0.8449	no
HI	Titrate	Hit	Core	Slope10: STGT=ST, Resp=Hit, N_T=Titrate	1.1459	0.035911	0.04712	7853	<.0001	1.0535	1.2383	yes	-1.0738	0.0137	0.0797	no
HI	Titrate	Hit	Core	Slope11: STGT=NT, Resp=Hit, N_T=Titrate	0.846	0.0126	0.2413	7853	0.0005	0.373	1.319	no	0.016117	0.9262	0.9972	no
HI	Titrate	Hit	Core	Slope12: STGT=GT, Resp=Hit, N_T=Titrate	0.2999	0.026666	0.4338	7853	0.4893	-0.5504	1.1503	yes	0.4164	0.3111	0.8449	no

Supplemental Table 3b1: Core stability analysis results for High Intake Core, ST and NT and GT, Titrate and NoTitrate, Hit and Miss, Baseline vs. Posttone: the Pval for slope vs. 0 represents the significance level for complete lack of stability (and evidence of tone-evoked changes Baseline vs. Posttone). If 1.0 did not fall within 95% of the lower and upper confidence values then the neuron was also considered to lack stability (and was also considered evidence of tone evoked change). The furthest right column is a comparison of Hit and Miss slopes summary of this data.

Int	Titration	HM	Region	Label	Slope	Intercept	Slope Std Error	DF	Pval Slope v 0	95% Conf. lower	95% Conf. upper	Tone Evoked?	Estimate	Pr>|t|	Adj P	Sig H v M
LI	NoTitrate	Miss	Core	Slope1: STGT=ST, Resp=Miss, N_T=NoTitrate	0.6653	0.005712	0.1152	10038	<.0001	0.4395	0.891	yes	-0.2269	0.3579	.	no
LI	NoTitrate	Miss	Core	Slope2: STGT=NT, Resp=Miss, N_T=NoTitrate	1.4054	-0.03193	0.1828	10038	<.0001	1.047	1.7637	yes	-0.7417	0.017	.	yes
LI	NoTitrate	Miss	Core	Slope3: STGT=GT, Resp=Miss, N_T=NoTitrate	Non-est	Non-est	Non-est	Non-est	Non-est	Non-est	Non-est					
LI	NoTitrate	Hit	Core	Slope4: STGT=ST, Resp=Hit, N_T=NoTitrate	0.4383	0.02999	0.2838	10038	0.1226	-0.1181	0.9947	yes	-0.2269	0.3579	.	no
LI	NoTitrate	Hit	Core	Slope5: STGT=NT, Resp=Hit, N_T=NoTitrate	0.6636	0.02726	0.2975	10038	0.0257	0.08048	1.2468	no	-0.7417	0.017	.	yes
LI	NoTitrate	Hit	Core	Slope6: STGT=GT, Resp=Hit, N_T=NoTitrate	Non-est	Non-est	Non-est	Non-est	Non-est	Non-est	Non-est					
LI	Titrate	Miss	Core	Slope7: STGT=ST, Resp=Miss, N_T=Titrate	0.7301	0.01859	0.2314	10038	0.0016	0.2766	1.1837	no	0.128	0.4288	.	no
LI	Titrate	Miss	Core	Slope8: STGT=NT, Resp=Miss, N_T=Titrate	0.9792	0.02356	0.1721	10038	<.0001	0.6417	1.3166	no	-0.5613	0.0069	.	yes
LI	Titrate	Miss	Core	Slope9: STGT=GT, Resp=Miss, N_T=Titrate	0.5098	0.02204	0.049	10038	<.0001	0.4137	0.6059	yes	1.3855	0.0249	.	yes
LI	Titrate	Hit	Core	Slope10: STGT=ST, Resp=Hit, N_T=Titrate	0.8582	0.01391	0.2356	10038	0.0003	0.3964	1.3199	no	0.128	0.4288	.	no
LI	Titrate	Hit	Core	Slope11: STGT=NT, Resp=Hit, N_T=Titrate	0.4178	0.01758	0.1135	10038	0.0002	0.1953	0.6403	yes	-0.5613	0.0069	.	yes
LI	Titrate	Hit	Core	Slope12: STGT=GT, Resp=Hit, N_T=Titrate	1.8953	-0.00741	0.6524	10038	0.0037	0.6164	3.1742	no	1.3855	0.0249	.	yes

Supplemental Table 3b2: Core stability analysis results for Low Intake Core, ST and NT and GT, Titrate and NoTitrate, Hit and Miss, Baseline vs. Posttone: the Pval for slope vs. 0 represents the significance level for complete lack of stability (and evidence of tone-evoked changes Baseline vs. Posttone). If 1.0 did not fall within 95% of the lower and upper confidence values then the neuron was also considered to lack stability (and was also considered evidence of tone evoked change). The furthest right column is a comparison of Hit and Miss slopes summary of this data.