Corticostriatal Action Selection in Rats With Opposed Attentional Biases

by

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Dedication

“To the Unsung Heroes: In honor of the rats whose every tiny step propelled me to the culmination of this research and whose tireless movements help unravel the mysteries of the brain.”

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Abstract

Moving across dynamic surfaces and obstacles requires integrating cues that guide movements, such as turning commands. The ability to detect and effectively integrate these cues, or the failure to do so (termed "misses"), can have severe consequences, leading to life-threatening falls and hospitalization. Prior research has highlighted the pivotal role of cholinergic modulation of fronto-cortical inter- and output neurons in detecting attention-demanding cues. This work hypothesizes that cues are transferred into the striatum via cortico-striatal glutamatergic (GLU) projections to integrate with striatal movement sequencing following cortical detection. The primary aim of this work was to investigate the role of glutamatergic cortico-striatal projections in enabling the initiation, sequencing, and updating of goal-directed actions. We investigated this hypothesis by recording GLU signaling in the striatal projection field of frontal cortical efferents, the dorsomedial striatum (DMS), in rats performing a task involving turn and stop-and-go signals. Leveraging the inherent attentional biases driven by varying cortical cholinergic capacities in goal-trackers (GTs) and sign-trackers (STs), this work aimed to illuminate the significance of corticostriatal glutamatergic signaling in complex movement control guided by cues. Amperometric recordings of GLU were time-locked to task events. Results revealed distinct increases in DMS glutamatergic concentrations, notably dissociated by phenotype and responses to task cues. Specifically, in GTs, detected turn cues evoked sharp, rapid increases in GLU activity, providing evidence of specific dorsomedial glutamate responses preceding and predicting the updating of movement sequences (turns). Conversely, glutamate responses in STs remained indistinguishable across different task responses. We further delved into the role of glutamatergic signaling in cue-triggered turning task (CTTT) performance by manipulating task parameters to increase demands on cue detection. We employed a dual-vector approach to silence corticostriatal projections to confirm the cortical origin of the GLU activity recorded in these experiments. Silencing of this pathway led to cue-triggered turning deficits and attenuated GLU increases in GTs, while STs remained largely unaffected. This

discrepancy indicates that STs, characterized by poor attentional control, may compensate for this deficit through bottom-up modulation of GLU release. However, this "replacement" mechanism may prove sufficient for well-practiced, cued movements but is expected to disrupt performance in unfamiliar and dynamic environments. Collectively, these findings shed light on distinct phenotypic variations in cue-guided behavior and have the potential to uncover the underlying neural mechanisms of attentional control in complex movement. Furthermore, delving into the impact of cognitive-motivational styles on corticostriatal glutamatergic signaling may yield invaluable insights into the progression and presentation of various disease states to pave the way for tailored, precise interventions.

Chapter I: Introduction

Many individuals navigate their daily routines, such as crossing the busy intersection near their workplace entrance, with little conscious effort or thought. Yet such seemingly simple everyday tasks require processing exteroceptive and interoceptive cues to facilitate adaptive corrections of ongoing actions, thereby selecting and planning movements that lead to goal-directed outcomes. For example, while crossing an intersection, a flashing *Don't Walk* signal may interrupt a preprogrammed walking sequence, and a misplaced step may evoke attentional shifts toward the detection of exteroceptive cues such as the status of the traffic light and the monitoring of body balance and the direction of forward motion, to correct action and prevent further movement errors, even falls. To ensure corrective action, attentional shifts may spotlight and prioritize cue-related information processing to guide corrective action (Mersmann et al., 2013; Yogev-Seligmann et al., 2012). The collected work of this dissertation aims to examine the underlying cognitive-motor mechanisms underlying action control in cue-guided movement. Furthermore, it explores how these mechanisms may differ across individuals with opponent cognitive-motivational styles. The results described in this dissertation may assist our understanding of a wide range of disease states that are influenced by such opponent cognitive-motivational styles, including deficient complex movement control and a propensity for falls in Parkinson's disease (PD) and the power of addictive drug cues to foster addictive drug seeking and relapse.

Attentional regulation of complex movement.

Approximately half of PD patients suffer from deficient complex movement control and a high risk for falls (Balash et al., 2005). These falls in PD patients have been associated with the loss of basal forebrain cholinergic neurons and attenuated attentional capacities (Allcock et al., 2009; Balash et al., 2005; Naismith et al., 2010; Wood et al., 2002). Past research demonstrated that reduced levels of cortical and thalamic acetylcholinesterase (AChE), which is indicative of cortical cholinergic terminal integrity, distinguish PD fallers

and non-fallers (Bohnen & Albin, 2011). In interaction with the disease-defining striatal DA depletion, the selection and filtering of cortical inputs are further disrupted (Guthrie et al., 2013; Strafella et al., 2005), yielding gait dysfunction and an elevated risk for falls. A rodent model has been developed to reproduce the dual dopaminergic and cholinergic, striatal and basal forebrain lesions seen in PD fallers (dual-lesioned, DL rats) and the resulting increase in the propensity for falls (Kucinski et al., 2017; Kucinski et al., 2013; Kucinski & Sarter, 2015). DL rats displayed high rates of falls associated with poor corrective actions, exacerbated by distractors, and, notably, fall rates were correlated with the impaired performance of DL rats in a sustained attention task (SAT). The SAT requires reporting of non-signal and signal events designed to instigate and necessitate attentional shifts of cue-primed states to cue-directed behavior to perform. Given the correlation between falls and attentional performance and correlations between cholinergic and dopaminergic losses and falls and attention, a heightened fall propensity in DL rats has been hypothesized to reflect a loss of compensatory attentional supervision of (impaired) striatal movement control. In other words, in PD patients with cortical cholinergic losses, movement error-evoked attentional shifts, customarily designed to facilitate the processing of exteroceptive cues, balance, and movement programming, are deficient, further depriving the striatum of cue-guided corrections and recovery of balance, and thereby further increasing the risk for major movement slips and falls. The sections of this chapter detail literature delineating the potential contributions of key regions/pathways in movement control: basal forebrain and cortical cholinergic systems in detecting environmental cues, cortico- and thalamostriatal connections in regulating action-outcome associations, and striatal cholinergic neurons as attentional-motor integrators.

Basal forebrain cortical projections and the cortical cholinergic mediation of the detection of cues.

An essential component in cue-guided behavior is the detection of cues. Detection here refers to a psychological – as opposed to a psychophysical – construct that entails elevated processing of exteroceptive or interoceptive cues so that such cues are integrated into the ongoing behavior and are capable of generating cue-directed responding and updating the system about the outcome of performing said response (Sarter et al., 2006). The sustained attention task (SAT) evaluates vigilance, requiring the

detection of infrequent signals over prolonged periods (McGaughy & Sarter, 1995). The task features high event rates, differing signal durations, variable intertrial intervals, complex response rules, and a distractor version to measure the capacity for task maintenance in the presence of a distractor and post-distractor performance recovery or, in short, the capacity for goal-directed or top-down control (Demeter et al., 2008; McGaughy & Sarter, 1995; Parasuraman et al., 1987). Lesioning of basal forebrain cholinergic cortical projections induces significant and specific impairments in the detection of cues, or "hits," while response rates in non-signal trials, or "correct rejections," remain unaffected (Baxter et al., 1996; Dalley et al., 2004; Martínez & Sarter, 2004; McGaughy et al., 2002; Newman & McGaughy, 2008; Paolone et al., 2013; Turchi et al., 2005). Performance on tasks not explicitly taxing attentional processes is minimally or not affected by basal forebrain lesions (Baxter et al., 1996; Chappell et al., 1998; Chiba et al., 1995; Frick et al., 2004). Correspondingly, elevated cortical ACh is observed only during the performance of tasks imposing explicit attentional demands (Arnold et al., 2002; Dalley et al., 2001; Himmelheber et al., 2000).

Consistent with the finding that cholinergic lesions selectively reduce hit rates, cue-evoked, phasic increases in frontal cortical acetylcholine (ACh) release, termed transients, are observed in rodents in SAT trials that begin with a cue and end with a hit but not during misses or non-signal trials. These cholinergic transients may instigate attentional shifts from cue monitoring to cue-guided behavior (Gritton et al., 2016; Howe et al., 2017). Supporting this hypothesis, optogenetic generation of cortical cholinergic transients during non-signal trials, when commonly no transients are observed, induces false reporting of signals. Conversely, inhibition of transients during signal trials increases the number of misses (Gritton et al., 2016). During SAT, local field recordings in the prefrontal cortex in animals reveal high gamma power during hits (Howe et al., 2017). These oscillations depend on cholinergic transients and are further enhanced during trials that necessitate an attentional shift (Gritton et al., 2016; Howe et al., 2017). Therefore, we hypothesize that this increased gamma power orchestrates cortical network synchrony and recruitment of subcortical regions, specifically the striatum, involved in movement selection and sequencing to generate cue-guided behaviors.

The dorsomedial striatum as the site of attentional motor integration.

As stated, attentional shifts may spotlight and prioritize cue-related information processing to guide corrective action and update responses. Therefore, following cholinergically mediated cue detection, the cortex may inform the striatum to guide and update ongoing movement for corrective action. The basal ganglia comprise interconnected nuclei vital to action control (Graybiel et al., 1994; Guthrie et al., 2013; Lanciego et al., 2012). The striatum, in particular, is associated with a myriad of cognitive, motivational, motor, associative, and sensorimotor functions (Gittis & Kreitzer, 2012; Hunnicutt et al., 2016; Lanciego et al., 2012; Ostlund et al., 2009). Within this dynamic circuitry, cortical glutamatergic innervation to the dorsomedial striatum (DMS), the focus of this dissertation, is pivotal to goal-directed behavior (Brasted & Wise, 2004; Corbetta & Shulman, 2002; Cox & Witten, 2019; Daniel & Pollmann, 2014; Featherstone & McDonald, 2004; Kim et al., 2013; Stalnaker et al., 2010). Its contributions encompass a spectrum of distinct yet intertwined functions such as temporal processing, motor learning, set-shifting, monitoring task completion, and action-outcome associations (Baker & Ragozzino, 2014; Bradfield et al., 2013; Cataldi et al., 2022; Emmons et al., 2017; Kim et al., 2013; Narayanan & Laubach, 2008; Ostlund et al., 2009; Vandaele et al., 2021b; Yin et al., 2005b).

Lesioning and/or inactivation of the DMS and/or of the corticostriatal pathway inhibits action-outcome learning and expression evaluated through outcome devaluation and contingency degradation manipulations (Hart et al., 2018; Kimchi et al., 2009; Morris et al., 2015; Yin et al., 2005a). Furthermore, blockade of dorsomedial striatal NMDA receptors also blocks action-outcome learning (Yin et al., 2005a). During cue-guided interval training, frontal cortex and striatal neurons exhibit monotonic ramping activity, and inactivation of the frontal cortex induces interval timing deficits that can be mitigated through corticostriatal stimulation, suggesting temporal processing by the frontostriatal circuitry (Emmons et al., 2017; Emmons et al., 2019). Similar ramp-like dorsomedial activity is found in rats performing lever press sequences with a rapid inverse in activity (inhibition-excitation and vice versa) upon reward port approaches, which may signal outcome evaluations (Vandaele et al., 2021a). On a related note, recordings from the dorsomedial striatum in animals engaged in a foraging task unveiled neural signaling that persisted from the response of one trial to the outcome of a response from the subsequent

trial (Kim et al., 2013). Human fMRI work suggests that frontostriatal circuitry may selectively gate updating only relevant working memory information to guide future behavioral responses (Chatham et al., 2014). These findings suggest that the corticostriatal pathway plays a significant role in temporal processing to link salient cue-guided action selection, execution of responses, and potential updating through outcome evaluation. Consequently, cortical cholinergic transients evoked during dynamic situations demanding attentional shifts may constitute the attentional aspect of attentional-motor integration within the striatum, thereby engaging or updating circuitry required to execute responses guided by past response-outcome rules.

Key candidates of this attentional-motor integration are striatal cholinergic interneurons. ChIs are the target of cortical and thalamic striatal afferents (Guo et al., 2015; Klug et al., 2018; Mamaligas & Ford, 2016). Acetylcholine (ACh) released from ChIs controls glutamate signaling from these afferents and inhibits output neurons (Ding et al., 2010; Zucca et al., 2018), suggesting that ChIs select corticostriatal inputs to guide movement shifts and corrections (Doig et al., 2014). Interactions between ChIs, corticostriatal, thalamostriatal, and nigrostriatal afferents are reciprocal (Chuhma et al., 2014; Kosillo et al., 2016; Maurice et al., 2015; Threlfell et al., 2012). These interactions position ChIs as an essential striatal integrator orchestrating complex movements and error-triggered corrections (Aoki et al., 2018; Apicella, 2017; Gritton et al., 2019; Tanimura et al., 2018). Removing thalamic innervation to ChIs prevents learning of changes to the contingency of a learned action-outcome association. Additionally, activation of thalamostriatal axons via salient stimuli presentation triggers ChIs spiking activity that suppresses the corticostriatal pathway's activity (Bradfield et al., 2013; Ding et al., 2010). Therefore, thalamostriatal innervation may modulate activity; acting through ChIs to facilitate updates to well-learned routines by inhibiting activity related previously learned rules mediated by corticostriatal inputs. As described in Avila et al. (2020), we previously demonstrated that chemogenetic inhibition of ChIs in the dorsomedial striatum induces turning deficits and high fall rates in otherwise intact rats and that stimulation of ChIs reduces falls and restores cued turning performance in DL rats (described above). Thus, these findings indicate ChIs as essential striatal integrators orchestrating complex movements and error-triggered corrections. Collectively, these findings suggest that ChIs integrate

specific contributions to acquisition, expression, and updating of action-outcome associations by thalamostriatal and corticostriatal projections for cue-guided complex behaviors. Relating to the work in this dissertation, we hypothesize that in dynamic scenarios requiring attentional shifts to enhance detection of movement-related cues, this information is “imported” to the striatum to engage and update circuitry required to execute responses. This dissertation aims to investigate the precise features of movement-related cues that might be “imported” by cortical glutamatergic afferents to the striatum. This exploration has the potential to provide insights into states of deficient complex movement control.

Animal models of opposing cognitive-motivational styles.

Individuals with Parkinson's disease who are prone to falls and outbred rodents displaying cognitive-motivational traits linked to addiction susceptibility (referred to as sign-trackers or STs) both exhibit impaired complex movement control (Balash et al., 2005; Kucinski et al., 2018). These rodents are classified as sign-trackers (STs), goal-trackers (GTs), or intermediates (INs) based on stimulus-reward learning measurements in PavCA or the Pavlovian Conditioned Approach Task (Flagel & Robinson, 2017). Over five consecutive sessions, these rodents experience repeated pairings of a banana-flavored sucrose pellet into a goal cup after the extension and subsequent retraction of an illuminated lever (conditioned stimulus, CS). Although all animals learn to obtain and consume the pellet, distinct phenotypes emerge based on cue-evoked behaviors: animals preferring the CS (STs), the goal cup (GTs), or oscillating between both (INs).

STs attribute incentive salience to the lever and other reward-predictive cues, actively approaching, interacting, and even working for access to these cues (Flagel & Robinson, 2017; Flagel et al., 2007; Meyer et al., 2012; Robinson & Flagel, 2009; Yager & Robinson, 2013). Their bias towards processing the motivational aspects of reward-related cues and the emergence of sign tracking depends on dopaminergic activity (Flagel & Robinson, 2017; Pitchers et al., 2017a). Notably, during abstinence periods when cues no longer predict rewards, STs display increased prefrontal dopamine signaling to these Pavlovian cues (Pitchers et al., 2017a). In contrast, GTs utilize these Pavlovian cues as indicators of reward availability (Holland, 1977; Meyer et al., 2012). GTs exhibit biases towards top-down processing reflective of their cognitive-motivational style, where they emphasize the

utility of cues to guide goal-directed behavior (Pitchers et al., 2017a). This is further supported by observations of cortical processing of higher-order contextual cues and the strong influence of occasion-setting cues to provoke responding in GTs (Bueno & Holland, 2008; Pitchers et al., 2017b; Trask et al., 2017). These differences in predictive versus discriminative stimuli driving behavior relate to the opponent cognitive-motivational styles of STs and GTs (Pitchers et al., 2017a)

"Cognitive-motivational styles" refer to predominant biases and cognitive-perceptual preferences that influence behavior (Sternberg & Zhang, 2014). In STs, their processing leans towards a strong inclination for "bottom-up" attention. Automatic, non-voluntary sensory processing, driven by the significance or nature of external stimuli, marks this form of attention (Connor et al., 2004; Kastner & Ungerleider, 2000; Kaya & Elhilali, 2014; Pinto et al., 2013; Theeuwes, 1994). Bottom-up processing of sensory or perceptual features of a given target cue may lead to the recruitment of "higher" cortical regions to execute behavior (Treisman & Gelade, 1980). Markedly, STs possess unresponsive neuronal choline transporters (CHTs), resulting in a limited capacity for cholinergic neuromodulation thought to underlie their predilection to bottom-up processing (Apparsundaram et al., 2005; Corbetta & Shulman, 2002; Koshy Cherian et al., 2017; Paolone et al., 2013). These processes may guide STs' performance in minimally taxing tasks but are ineffectual upon increased demands (Kucinski et al., 2018; Paolone et al., 2013; Phillips & Sarter, 2020). They exhibit fluctuating performance in tasks with high attentional demand, particularly in later blocks of the SAT, indicating potential vigilance decrements (Paolone et al., 2013). Additionally, their performance deteriorates when faced with a distractor, and they struggle to recover in post-distractor periods. Furthermore, STs experience higher fall rates compared to GTs in conditions that demand intricate cognitive control for maintaining balance, necessitating vigilant monitoring and precise adjustment of forelimb placements to prevent slips and falls (Kucinski et al., 2018). These findings suggest that this deficiency in complex movement control in STs may stem from an absence of effective top-down attentional regulation.

In contrast, GTs engage top-down planning and timing of forward movement, indicating stronger vigilance, and are sensitive to outcome updates as seen with changes to contingencies or devaluation (Kucinski et al., 2018; Morrison et al., 2015; Paolone et al.,

2013). Top-down attention is voluntary control which relies on higher-order cognitive processes, including internal goal and past knowledge representations (Kastner & Ungerleider, 2000). Frontal and parietal cortices regions implicated in governing the encoding of goal-oriented information exhibit robust activation during tasks that heavily challenge top-down processing (Banerjee et al., 2017; Howe et al., 2013a; Vanunu et al., 2021). Prioritizing relevant information may occur through enhanced feature-based processing (Maunsell & Treue, 2006; Reynolds & Chelazzi, 2004; Vanunu et al., 2021), improved discriminability (Baluch & Itti, 2011; Reynolds & Chelazzi, 2004), lower thresholds for neural responsiveness (Baluch & Itti, 2011; Lauwereyns et al., 2002), and promoting synchronization of neuronal populations that suppress firing in response to irrelevant stimuli (Bahmani et al., 2019; Börgers et al., 2008; Cohen & Maunsell, 2009; Howe et al., 2017; Paneri & Gregoriou, 2017).

As discussed above, cortical cholinergic transient activity is necessary for detecting cues that facilitate and guide external cue-driven behavior. We propose that successfully navigating complex, dynamic environments requires attentional shifts and cue detection, both mediated by cortical cholinergic signaling. Therefore, in this work, we proposed to capitalize on these animals of opponent cognitive-motivational styles secondary to differing cortical cholinergic capacities. We hypothesized that during a cue-triggered turning task; only GTs would exhibit cue-locked increases in dorsomedial glutamatergic concentrations secondary to cortical cholinergic transient activity. Investigating potential differences in corticostriatal glutamatergic signaling between individuals with opposing cognitive-motivational styles may yield valuable insights into the development and manifestation of various disease conditions, especially those characterized by significant losses in specific neuronal populations.

Strategy and limitations.

The work presented in this dissertation aims to address if glutamatergic cortico-striatal projections enable the planning and sequencing of goal-directed actions. We choose to capitalize on the inherent cognitive-motivational biases driven by differing cortical cholinergic capacities in GTs and STs to investigate the role of corticostriatal glutamatergic signaling in cue-directed, complex movement control. Therefore, we conducted awake and behaving recordings of GTs and STs performing a cue-triggered

turning task (CTTT) with precise manipulations of neural regions implicated in cue detection, and task parameter manipulations thought to increase the cognitive demands of the CTTT. There are several limitations regarding the methodology in the work presented here. This work does not directly measure or evoke glutamatergic release through electrical stimulation or potassium-evoked release. Additionally, the sample sizes reported here are low due to use of a singular recording system. Future work characterizing glutamate concentrations in GTs and STs performing in the SAT could address the limitations listed here.

Summary of presented works.

Chapter II examines the differences in glutamatergic (GLU) concentrations in the dorsomedial striatum in animal models of opposing attentional styles mediated by unresponsive versus elevated levels of cortical cholinergic signaling, STs, and GTs, respectively. We proposed that GLU signaling in the striatum tied with cue presentation in cue-triggered turns reflects upstream cortical signaling. Only cues preceding and predicting situations that require updating movement sequences (turns) elicited dorsomedial GLU in GTs instead of stop-and-go sequences (stops). In contrast, GLU in STs was indistinguishable across responses.

Chapter III investigates the importance of glutamatergic signaling in cue detection thought to underlie CTTT performance. We manipulated parameters to increase the cognitive demand of the CTTT. The frequency or proportion of trial types was increased or decreased in separate animals by manipulating the inter-trial interval (ITI) or ratio of turn and stop cue presentations. Extending the ITI increased cue-triggered turn rates in GTs without affecting GLU concentrations. In contrast, shortening the ITI attenuated GLU levels without affecting the rate of cued turns in GTs. In STs, a shortened ITI resulted in increased cue-triggered turning. No behavioral effects or effects on GLU concentrations were found from varying the proportion of trial types in GTs or STs.

Chapter IV delves into the necessity of cortical inputs to the striatum in GTs and STs performing the CTTT. We proposed that dorsomedial striatal GLU transients originate from corticostriatal projections to drive cue-guided movement. The chapter examines the effects of silencing the corticostriatal pathway using a dual-vector chemogenetic

approach on GLU transients and CTTT performance in GTs and STs. Corticostriatal silencing resulted in cue-triggered turning deficits and attenuated GLU transients, specifically in GTs. Additionally, transfection efficacy in the prelimbic area correlated with the severity of behavioral deficits.

Collectively, the work presented here provides insights into the neural mechanisms and the significance of corticostriatal glutamatergic signaling underlying goal-directed action in complex movement control. By examining the impact of cognitive-motivational styles on corticostriatal glutamatergic signaling, we may elucidate new avenues for understanding, diagnosing, and treating differing disease states, ultimately leading to more individually targeted and effective interventions.

Chapter II: Differential Striatal Glutamatergic Signaling Elicited by Movement Cues in GTs and STs.

Abstract

Moving across dynamic surfaces and obstacles requires integrating cues that guide movements, such as turning commands. Failures to detect and integrate such cues (i.e., misses) can lead to life-threatening falls and hospitalization. Cholinergic modulation of fronto-cortical inter-and output neurons was previously demonstrated to mediate the detection of attention-demanding cues. We hypothesize that such cues are transferred into the striatum via cortico-striatal glutamatergic (GLU) projections and integrated with striatal movement sequencing. We investigated this hypothesis by recording GLU signaling in the striatal projection field of frontal cortical efferents, the dorsomedial striatum, in rats performing a task involving turn and stop-and-go signals. Moreover, we observed real-time GLU signaling in rats classified as sign-trackers (STs) and goal-trackers (GTs). Since STs were previously shown to exhibit relatively poor cortico-cholinergic attentional control, we predicted that turn cues are missed more frequently than in GTs and, if utilized, evoke truncated GLU activity. Amperometric recordings of GLU were time-locked to task events. In GTs, detected turn cues were associated with sharp increases (10-17 μM increases over baseline) in GLU activity and peaking within 250-350 ms from cue onset. Missed cues did not evoke such transients in GTs. Comparable GLU transients during detected and missed turn cues were seen in STs. Together, these results indicated that GTs execute cued turns and avoid misses through cortico-striatal integration of cues. STs may perform these well-practiced trials by utilizing more habitual response patterns, less dependent on the cortical detection of cues and subsequent integration into striatal circuitry.

Introduction

Hurrying to catch your usual bus after a tiring workday, you step off the curb at a busy 4-way intersection. Your foot slips on an unexpected object; your eyes dart below to see a crushed beer can unceremoniously discarded from a St. Patrick's Day party. You quickly reposition your limbs, regain your balance, double-check for approaching cars, and resume crossing the intersection. When faced with disruptions to familiar, well-practiced actions, attentional shifts towards detecting relevant cues (the crushed can) and towards corrective actions (repositioning limbs) to update and reprogram movements (resuming walking) are essential to prevent life-threatening consequences. Although cortical cholinergic-mediated detection of environmental cues has been extensively investigated, the subsequent recruitment of subcortical regions to execute goal-directed responses, as well as the specific aspects of these cues that are transmitted or "imported" into these subcortical regions, such as the striatum, warrant further investigation (Gritton et al., 2016; Howe et al., 2013b; Parikh et al., 2007). To address this, we conducted recordings in the striatal projection field of frontal cortical efferents, the dorsomedial striatum, in animal models of low versus high capacity cortical cholinergic input systems (sign-trackers "STs" and goal-trackers "GTs") performing a task involving turn and stop-and-go signals.

Cholinergic basal forebrain projections are essential in successful cue detection (Berry et al., 2015; Berry et al., 2014; Gritton et al., 2016). Supporting this, immunotoxin-induced lesioning of basal forebrain cortical cholinergic afferents in rats performing a task requiring reporting signal and non-signal events over a prolonged time (SAT) resulted in selective deficits to reporting signals or "hits," while non-signal reporting or "correct rejections" were intact (McGaughy et al., 1996). Consistent with these results, right prefrontal cortical amperometric recordings of rodents performing the SAT revealed trial-by-trial selective phasic cholinergic activity termed "transients" (Parikh et al., 2007). Cholinergic transients were present during trials resulting in hits after a prolonged temporal delay or perceived non-signal trials. Therefore, these transients may mediate cue detection in scenarios requiring a shift from cue monitoring or perceptual attention to cue-directed responding, dubbed "attentional shifts" (Howe et al., 2013b; Howe et al., 2017). Here, "cue detection" refers to enhanced cue processing to enable these cues to trigger responses corresponding with pre-established stimulus-response rules (Posner, 1980).

Subsequent experiments exploring the causal role of this activity highlighted the necessity of these transients in cue detection as suppression of endogenous cholinergic activity decreased hit rates (Gritton et al., 2016). In addition, optogenetic generation of cholinergic transients was sufficient in provoking false reporting of signals or “false alarms.” A proposed mechanism underlying how cortical cholinergic transients mediate signal detection involves generating, synchronizing oscillations, and transmitting this coordination across cortical and subsequent subcortical regions to execute complex, cue-oriented behavior (Howe et al., 2017). Navigating dynamic environments, particularly upon disruption of motor sequences, may require attentional shifts to detect exteroceptive and proprioceptive cues and initiate necessary corrections. Given the role of cholinergic transients in attentional shifts to aid cue detection, structural and functional abnormalities in prefrontal cortical cholinergic signaling may result in ineffective complex cue-initiated action.

Sign-trackers (STs) and goal-trackers (GTs) display opposing biases to cues predictive of reward availability thought to result in part from relatively unresponsive versus responsive cortical cholinergic systems (Koshy Cherian et al., 2017; Meyer et al., 2012) Precisely, K^+-evoked ACh release in GTs was higher than in STs, mirroring lower levels of cortical extracellular ACh in SAT-performing STs (Koshy Cherian et al., 2017; Paolone et al., 2013). This work demonstrated that ineffective CHT-mediated choline transport in STs imposes limited capacity for cortical cholinergic modulation. Consequently, these findings suggest that cholinergically mediated cue detection in STs is disrupted or even absent, thereby underlying variable SAT performance and deficient complex movement control (Kucinski et al., 2018; Paolone et al., 2013). Specifically, in a complex motor control task, STs fail to detect relevant exteroceptive and interoceptive cues, such as slips and improper limb placements preceding falls.

We hypothesized a task requiring processing and integration of movement-related cues to guide behavior would evoke cortical cholinergic activity, and that this cue-related information in this task is transferred through corticostriatal glutamatergic (GLU) projections and then integrated with striatal movement sequencing. Here, we performed amperometric recordings via glutamate-sensitive biosensors in the dorsomedial (DMS) striatum of GTs and STs performing the cue-triggered turning task (CTTT). This task

requires distinct responses (turns or stops) to two cues of differing modalities. We predicted that in STs given relatively poor cortico-cholinergic modulation, task cues are missed more frequently than in GTs and, if utilized, evoke truncated GLU activity. In GTs, we found robustly higher increases of cue-locked GLU concentrations during turn trials that resulted in a turn compared to missed turns and cued stops. GLU levels in STs did not differ between these trial types or the response of the animal. Conversely, GLU concentrations time-locked to the reward delivery were higher in STs than in GTs. These findings suggest that in GTs, dorsomedial striatal GLU increases only in situations requiring reprogramming and updating of action plans. In contrast, elevated dorsomedial GLU in STs during reward delivery may reflect subcortical modulation of cortical or thalamic terminals. These findings highlight patterns of dorsomedial striatal glutamate across phenotypes and behavioral responses, thus implicating potential cognitive and neural processes guiding CTTT performance explored in later chapters.

Methods

Subjects. 378 Sprague Dawley rats (obtained from Inotiv, West Lafayette, IN, and Taconic, Rensselaer, NY; *n* = 215 females; 250-500 g) were individually housed on a 12-hour light/dark cycle (lights on at 7:00 AM) at ~21ºC with *ad libitum* access to food (Laboratory Rodent Diet 5001, LabDiet) and water. The experiments detailed below used four separate cohorts of rats (each consisted of *n* = 79 rats, 39 females). The cohorts were composed of rats obtained from both Taconic and Inotiv. All rats underwent amperometric recordings; cohorts were obtained and formed across 19 months, reflecting the limited capacity for amperometric recordings. The remaining animals were used for other experiments. All experimental procedures were approved by the University Committee on the Use and Care of Animals (UCUCA) at the University of Michigan and carried out in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care. Animals acclimated to housing quarters for two days before the onset of experimental procedures.

Pavlovian Conditioned Approach (PCA) apparatus and training. Animals were trained in standard operant chambers (Med Associates Inc., 20.5 cm long x 24.1 cm wide x 29.2 cm tall) contained within sound-attenuating boxes (Fig. 2.1a). Each chamber was equipped with a fan, a red house light, a single retractable illuminated lever, a goal cup,

and a pellet dispenser. The house light was opposite the wall with the goal cup and lever attached. The pellet dispenser was attached to the goal cup, located 2.5 cm above the stainless steel rod floor. The grid floor (4 cm from the chamber bottom) was suspended above a catch tray filled with size corncob bedding. One 45 mg banana-flavored sucrose pellet (Bio-Serv) was dispensed every trial. Goal cup entries were marked by each break in the infrared beam located approximately 1.5 cm from the base of the cup. An extendable lever with a white LED was located 6 cm above the floor and either to the goal cup's left or right (counterbalanced between chambers). A 15-g force was required to detect lever contacts. Med-PC software (version IV) executed the sequence of events and collected lever press and magazine entry data. This data included the number of lever presses, latency to the first lever press, number of goal cup entries during lever extension and during the inter-trial interval, the latency to the first goal cup entries, and total number of entries/nose-pokes. Before PCA training, animals were handled for 4 days and given 15 banana-flavored pellets daily to prevent neophobia. On the first day of PCA training, animals were placed into the operant chambers to acclimate to the pellet delivery into the goal cup. The red house light illuminated after a 5-minute habituation period, and then 25 trials consisting of single pellet deliveries on a VI-30 schedule occurred over approximately 15 min. Over the next 5 consecutive days, 25 lever-pellet paired presentations occurred on a VI-90 schedule over about 37.5 min. In each trial, the illuminated lever extended into the chamber for 8 s, and then upon retraction, a pellet was delivered into the goal cup.

PCA screening index. Based on their PCA index score, animals were classified as goal-trackers (GTs), intermediates (INs), or sign-trackers (STs). The PCA index averages three approach measures: response bias, latency, and approach probability (Howe et al., 2015; Meyer et al., 2012; Phillips & Sarter, 2020). Response bias was calculated as the difference between the total lever presses and total goal cup entries over the total number of responses [(lever presses - goal cup entries) / (lever presses + goal cup entries)]. Latency was determined by the difference between the latency to approach the goal cup and the latency to approach the lever during conditioned stimulus (CS) presentation over the 8-second period the lever was extended [(goal cup latency – lever latency) / 8]. Approach probability indicated the likelihood of lever presses or goal cup entries. It was

calculated as the difference between the number of trials with lever presses during CS presentation out of 25 trials and the number of goal cup entries during that same time window. The index ranged from -1.0, indicating the maximal amount of goal cup approaches and contacts, to 1.0, indicating the maximal amount of approaches and contacts to the lever. The averaged values from Days 4 and 5 generated a score to classify each rat into three phenotypes. The cutoff range for STs was 0.5 to 1.0, biasing toward lever-directed behavior (Fig. 2.1b). The cutoff range for GTs was -0.5 to -1.0, favoring goal cup-directed behavior (Fig. 2.1c). Animals with scores between these two ranges were classified as INs, with a score of 0 indicating similar approaches and contacts to the lever and goal cup.

Cue-triggered turning task (CTTT) apparatus. The 32.51 cm wide by 153.67 cm long treadmill was constructed by modifying a 2200 Series Flat Belt End Drive Dorner conveyor (Dorner, WI, Fig. 2.2a). The conveyor belt surface material was polypropylene, friction-resistant, and easily cleaned with ethanol or soap. The conveyer was paired with a Dorner Variable Speed Controller (Dorner, WI), with speeds ranging from 0 cm/s to 32 cm/s or 19.2 m/min. The Variable Speed Controller included a reversing controller, allowing the belt to rotate clockwise, "forward," or counterclockwise, "reverse." An in-house-made Faraday cage was placed on top of this conveyer, 145.10 cm long, 32.51 cm wide, and 38.1 cm tall. The wooden frame of the Faraday cage was enclosed by a woven copper mesh and grounded to block out static electric fields. Plexiglass inserts were placed inside the Faraday cage to prevent rodents from chewing on the mesh. Experimenters raised two wooden panels on the cage to place rodents on the treadmill. The 28V DC, 100 mA stimulus light was approximately 2.54 cm in diameter with a flat lens and mounted on both sides lengthwise on the Faraday cage (MedAssociates, Inc., St. Albans, VT). The auditory cue was a Mallory-SonAlert audible device. This 28V DC, 18 mA device was mounted on the center of the Faraday cage and was approximately 4.3 cm long by 4.3 cm wide by 3.6 cm tall. This device emitted a continuous tone at 68 dBA sound pressure level, which is within the acceptable range for chronic presentations, to neither elicit a fearful response nor induce hearing loss (Castelhano-Carlos & Baumans, 2009; Turner et al., 2005). These devices were controlled by a MedAssociate interface and a relay module to run our custom program. Briefly, rats were trained to walk

on a treadmill until the onset of a “stop” or a “turn” cue, following which the treadmill restarted in the same or the reverse direction, respectively. As illustrated in Figure 2b, the cue is presented for 2 s, and 1 s later; the treadmill pauses for 5 s. Rats learn to respond to one cue to stop and the other to turn, and then the treadmill restarts in the same or opposite direction (Avila et al., 2020). Performance sessions were videotaped with four web cameras (Logitech C920x HD Pro Webcam, Full HD 1080p/30fps) and relayed to an Intel Xeon workstation (Dell, Round Rock, TX) via USB cords and processed with OBS Studio (Open Broadcaster Software, free and open source software).

Training regimen. Rats underwent a treadmill acclimation regimen adapted from (Arnold & Salvatore, 2014). Rats were handled for 20 min for two days before exposure to the treadmill. On the second day, rats were placed in the Faraday cage for 15 min with the belt paused. All sessions were conducted under dim red lighting to minimize stressors and increase the discriminability of the light cue. For a week, rats were placed into the Faraday cage for 3 min with the belt paused and then trained to walk on the treadmill at speeds up to 9.6 cm/s or approximately 6 m/min. The experimenter manually set the initial rate and direction of the treadmill, with the initial direction counterbalanced within and across subjects. During this training, rats were reoriented by gentle prodding if they began to walk in the opposite direction of the belt. Upon each session completion, rats were immediately returned to their home cage and housing room. Between sessions and subjects, the Plexiglass walls of the Faraday cage and the treadmill belt were wiped down with 70% ethanol and soap.

Table 1 details the training regimen. The initial treadmill speed was set at 3.2 cm/s for the first two days and gradually increased by 1.6 cm/s every 2 min. Each session lasted 20 min, so the ending speed was 8 cm/s. On the third and fourth days, the initial rate was set at 6.2 cm/s, increasing by 1.6 cm/s every 5 min until the maximum speed for the training was 9.6 cm/s. On the fifth and sixth day, rats walked at 8.0 cm/s for 10 min and 9.6 cm/s for the last 10 min. Finally, on the seventh day of this training regimen, rats walked at 9.6 cm/s for 20 min. In the next training phase, rats were placed on the treadmill and presented with either an auditory or a visual cue.

Contrary to the acclimation training phase, during which experimenters manually controlled the treadmill, this phase was controlled entirely by custom scripts using Med-

PC software and interface (MedAssociates). As described above, rodents walked at a speed of 9.6 cm/s. The cues were presented for 2 s, and cues of the same modality were presented at a maximum of twice a row. The intertrial interval (ITI) was 60±30 s. The total number of trials per daily session was 18, with a maximum duration of a test session of 20 min. Rats underwent cue training for approximately two to three weeks. Then, upon reaching the performance criterion, defined as 70% cued responses to both cues for two consecutive days, rats were tested and videotaped for four days to establish baseline performance for each animal. Turns were considered cued if they occurred before the restart of the treadmill belt. After that, rats underwent surgery for chronic microelectrode array implantation.

CTTT performance measures. Performance measures (turns, missed turns, stops, false turns) were extracted from offline scoring of session videos. For a successful turn to be scored, the animal must have initiated a turn, defined by a rotation of the longitudinal orientation of the body of at least 90°, before the treadmill restart, that is, within 8 s of the cue onset (see Fig. 2.2b). A stop was defined as a cessation of forward movement. For stops occurring before the treadmill stops, rats would typically stop while positioned at the backend of the treadmill so that following a stop, the treadmill would transport them to the front of the treadmill (within the remaining 1-3 s until the treadmill stopped), without the rat contacting the front end of the test chamber. Following the treadmill stop and during the 5-s pause, a false turn was scored if the animal (falsely) turned. Animals were rewarded with a banana pellet for successful responses (cued turns and stops only). Turn and stop scores were expressed as a ratio of the number of trials/sessions that began with turn or stop cues. The ratio of both turns and stops were averaged for overall performance scores on the CTTT (turns/turn trials + stops/stop trials/ total trials); CTTT score). Furthermore, the total number and proportion of cued responses executed during the period starting with the cue onset and until the treadmill stopped were separately analyzed to obtain a measure reflecting immediate, cue-evoked responses. Additional performance measures were extracted from training and testing sessions to characterize further individual performance variability and phenotype-related differences. These measures included days to reach criteria, performance level, and variability. The

performance level was calculated for turns and stops. Performance variability was defined as the standard deviation of the performance of an individual rat across sessions.

Amperometric recordings of glutamate currents. Extracellular glutamate concentrations were measured by oxidizing glutamate on an electrode surface featuring immobilized glutamate oxidase (GO) and by oxidizing the resulting peroxide levels using amperometry. Based on a calibration curve generated in vitro, the resulting currents can be expressed as the glutamate concentration at the recording site (Parikh et al., 2010; Parikh et al., 2007). The construction of glutamate sensors and the amperometric measurement scheme were detailed previously (Koshy Cherian et al., 2017; Parikh et al., 2010; Parikh et al., 2007; Parikh et al., 2008). The configuration consisted of a ceramic backbone probe (Quanteon) housing four recording sites crafted from platinum-iridium (Pt/Ir). These recording sites were systematically grouped into two pairs, separating 30 μm between each member of a pair and a 100 μm spacing between the pairs. Each recording site measured 15 × 333 μm in dimensions. Furthermore, these recording sites were linearly arranged along the shank of the probe (Fig. 2.3c). Microelectrode arrays were modified for in vivo awake and behaving recordings by soldering four enamel-coated magnet wires (30 ga) to the terminals on the electrode panel and gold-pin connectors. Reference electrodes were constructed by soldering Ag/AgCl reference electrodes prepared from 0.008” silver wire (A-M Systems, Carlsberg, WA) to gold-pin connectors. The pins were then inserted into a 9-pin ABS plug (GS09PLG-220, Grinder Scientific) and adhered to the microelectrode with epoxy (Parikh et al., 2007). Custom 9-pin ABS plugs were also printed with a STUDIO G2 3D printer (BigRep, Berlin, Germany).

The top pair of electrodes, active sites, were coated with a GO (Sigma-Aldrich) solution cross-linked with a BSA and glutaraldehyde mixture by manually applying microdroplets using a 1-μl Hamilton syringe (Fig. 2.3d,e). The lower pair of electrodes were coated with only the BSA and glutaraldehyde mixture, termed sentinel sites, to isolate currents unrelated to glutamate. After a minimum 24-hr incubation period to ensure optimal adherence of the enzyme layer, an exclusion layer composed of m-(1,3)-phenylenediamine (mPD) was electroplated onto each recording site's surface by 5-min application of 0.85 V versus an Ag/AgCl reference electrode (Bioanalytical Systems). This exclusion layer prevents sensing electroactive interferents such as ascorbic acid (AA) and

catecholamines (Donovan et al., 2022). After another 24-hr incubation period for the electrode sites to dry, recording sites were calibrated to determine the sensitivity for glutamate, selectivity for glutamate versus interferents, stability, and limit of detection of glutamate. Calibrations were conducted using a FAST-16 electrochemical system (Quanteon). A constant voltage of 0.7 V was applied versus an Ag/AgCl reference electrode, and the system was placed in a heated 40 ml bath of 0.05 M PBS. Following a 30-minute baseline recording, a solution containing 20 mM ascorbic acid (AA, interferent), 20 mM glutamate (analyte), and 2 mM dopamine was introduced to the bath. These additions generated the following concentrations: 250 µM AA, 20, 40, and 80 µM glutamate, and 2 µM dopamine. Changes in amperometric current at individual electrode sites were measured after each solution to calculate the slope (sensitivity), the limit of detection, selectivity for AA and dopamine, and linearity (R^2). Glutamate sensors were required to have a sensitivity of >5 pA/µm, a limit of detection <1.0 µm glutamate, glutamate:AA selectivity ratio >50:1, and a linear response to increasing glutamate concentrations (20-80 µm glutamate) of R >0.95.

Chronic implantation of microelectrode arrays (MEAs). Upon reaching the criterion in the CTTT (≥70% cued turns and stops for two consecutive days), microelectrode arrays were chronically implanted into the dorsomedial striatum. Rats were anesthetized using isoflurane gas (5% induction and 1–3% maintenance) and mounted on a stereotaxic frame on a heating pad to maintain a 37°C body temperature. Ophthalmic ointment lubricated eyes. A craniotomy and durotomy using a bent 27-gauge needle for dura removal were performed above the right dorsomedial striatum (AP: +0.50 mm; ML: −2.20 mm from bregma). Three stainless steel screws were threaded into the cranium. The prepared microelectrode probe was then lowered 4.5 mm dorsoventrally from the dura into the striatum (Fig. 2.3b). At the same time, an Ag/AgCl reference electrode was implanted at a remote site in the contralateral hemisphere. The microelectrode assembly was anchored with methyl methacrylate dental cement, and exposed regions of the skull were filled with a translucent, medium-viscosity silicone adhesive to minimize leakage of the dental cement onto the brain. Animals rested over a 48-hour recovery period before moving to the next phase of the experiment. Amperometric recordings were collected by connecting the head stages to a FAST-16 potentiostat/data electrochemical system via a

shielded cable and low-impedance commutator (Fig. 2.3a). Glutamate sensing was accomplished through GO catalyzation of the conversion of glutamate into hydrogen peroxide and glycine betaine. The hydrogen peroxide by-product was electrochemically oxidized by applying 0.7 V versus the Ag/AgCl reference electrode and digitized at a sampling rate of 5 Hz. Behavioral sessions began following a 50-minute baseline period.

Verification of microelectrode array placements in the dorsomedial striatum. Microelectrode arrays (MEAs) were chronically implanted into the dorsomedial striatum to record extracellular glutamate levels while performing the CTTT. As described in Kucinski et al. (2022), following the completion of experiments, we injected rats with a lethal dose of sodium pentobarbital (270 mg/kg, i.p.) and then rats were transcardially perfused with saline, followed by 4% paraformaldehyde in 0.15 m sodium-phosphate solution, pH 7.4. Brain extraction followed transcardial perfusions, and brains were postfixed in 4% paraformaldehyde for 24 h, then submerged in 30% sucrose solution until they sank. Using a freezing microtome (CM 2000R; Leica), 35-µm thick brain slices were sectioned and stored in cryoprotectant until further histologic processing. Sections were mounted and processed with a Cresyl Violet Nissl stain to confirm MEAs placement. A Leica DM400B digital microscope was used to photomicrograph the sections at 1.25X and 5X magnification at three A-P levels (0.2 mm, 0.5 mm, and 1.0 mm). The locations of microelectrodes tips were within pre-defined limits in the dorsomedial striatum, confirmed histologically.

Data processing and analysis of glutamate currents. Data processing was achieved through a custom Matlab script. The background current recorded on sentinel sites was subtracted from the glutamate-specific current recorded on the active sites (Fig. 2.3f-h), normalized by dopamine response, and then converted to glutamate concentration based on calibration measurements. Glutamate concentration was analyzed over 13 s bins, 3 s before cue presentation through the restart of the treadmill belt (10 s). The concentration of glutamate currents 2.5 s, approximately 10 data points, before cue presentation served as a baseline for each trial for analysis. Changes from the baseline were used in statistical analyses (Parikh et al., 2007). The peak amplitude (µM), onset, time to peak, and number of peaks were extracted for each trial. The time to peak was defined as the time for the current to reach peak amplitude. PeakDet, which requires setting a threshold (red dotted

line (Fig. 2.3i) and minimum difference, was utilized to identify peaks accurately. For our purposes, a value must be greater than the set threshold to be considered for peak identification, which was 3 standard deviations above the baseline (2.5 s before cue presentation) determined for each trace. PeakDet identifies a peak as the highest point with a minimum difference (μm for our analysis) to points on both sides of the peak (Ge et al., 2016; Ghosh et al., 2022).

Statistical analyses. Statistical analyses were performed using SPSS for Windows (version 28.0; SPSS) with α set to 0.05. Fisher's exact tests were used to test distributions. Given the complexity of the experimental design (effects of phenotype on turn and stop rates elicited by turn and stop cues of either modality), here we first determine potential differences in GLU levels across trial types and response categories within the individual phenotypes. Given the within and between-group (potentially unequal group sizes) experimental design detailed throughout the work presented, we determined that linear-mixed effects models (LMMs) would be the most appropriate statistical method to analyze collected data. One key issue in determining which method to apply was how to define the sample size; in these experiments, our sample size may be the total number of animals or the multiple individual traces collected for each animal. Many statistical models rely on the assumption that observations/measurements (animals or traces) are independent from one another (Cruz et al., 2020). Defining sample size in these experiments is a complex issue as the repeated measurements from each animal across days may or may not be distinct (Lazic et al., 2018). This concept of repetition of distinct units with the same manipulation or treatment is replication. However, repeated measurements may contribute pseudoreplication, increasing the chances of Type 1 errors.

The benefits of LMMs regard the variance-covariance structure of the data. LMMs do not assume that observations are independent (Magezi, 2015; Schielzeth et al., 2020; Yu et al., 2022). In addition, tests such as repeated-measures ANOVAs require compound symmetry or sphericity to hold (Magezi, 2015). Compound symmetry refers to the same variability of measurements across groups (homogeneity of variance), and any changes due to the experimental manipulation(s) between groups must be consistent (similar covariances). However, if compound symmetry is violated, then sphericity or equal

variances of the measurements across all groups but not covariances must hold. Common corrections such as Huyn-Feldt or Greenhouse-Geiser may be used but are conservative. Repeated-measures ANOVAs also require complete data sets, which can lead to excluding subjects with missing sessions/measurements. With LMMs, effects fall under two categories: fixed effects, fixed but unknown coefficients of the manipulations with specific levels set by an experimenter and explanatory covariates, and random effects, levels from random sampling such as subject effects that account for variability in individual observations and the interactions between all effects.

To analyze PCA behavior, CTTT performance measures, and electrochemical data, we employed linear mixed-effects models (LMM) with restricted maximum likelihood estimation. For PCA analysis, the 5 sessions of PCA were used as a repeated fixed effect, and phenotype, vendor, and cohort were used as the between-factors fixed effects with a subject identifier random intercept. For CTTT performance measures, repeated sessions were used as the repeated fixed effect, and phenotype was used as the between-subjects fixed effects, with a subject identifier random intercept. The covariance structures with the lowest Akaike's information criterion were selected for each model (Verbeke, 1997). If LMMs reported significant interactions, repeated measures ANOVAs were used to compare sessions against each other with Huynh–Feldt-corrected F values and corrected degrees of freedom used in case of violation of the sphericity assumption. Exact p values were reported, and effect sizes (partial eta squared) were indicated for major results derived from Bonferroni pairwise comparisons of estimated marginal means (Greenwald et al., 1996). Data graphs depict individual values, estimated marginal means (EMMs), and standard error of the mean (SEM).

Results

GTs and STs screening. PCA screening yielded 113 (30%) GTs, 155 (41%) INs, and 110 (29%) STs. Figure 2.4 shows the distribution of PCA scores across the five test sessions for the 79 rats eventually classified as GTs, STs, or INs (29 GTs (13 females), 27 INs (13 females), and 22 STs (13 females). These rats were used for CTTT training and amperometric recordings. Subgroups of rats were formed, counterbalanced by sex and phenotype, to assess the effects of experimental manipulations.

PCA scores did not significantly differ between the four cohorts (no main effects of cohort on response bias (respbias), probability difference (probdiff), latency, or index; $F < 1.74$, $p > 0.16$, for all). Chi-square tests determined if sex or the commercial source of the rats (vendor) influenced the distribution of phenotypes. There was a significant difference in the distribution of PCA indices between the two vendors. Rats from Inotiv displayed a bias towards sign tracking (lever-directed behaviors), while rats supplied by Taconic showed a bias towards goal tracking (goal cup-directed behaviors: $X^2(2, N = 378) = 36.62$, $p < 0.001$; Fig. 2.5a). No significant sex effects on PCA behavior (respbias, probdiff, latency, index) nor the sex-specific distribution of phenotypes for either vendor were found (Inotiv: $X^2(2, N = 133) = 1.30$, $p = 0.52$; Taconic: $X^2(2, N = 245) = 1.41$, $p = 0.50$; Fig. 2.5b,c). GTs and STs were selected from each vendor after PCA screening and combined to balance the distribution represented in the experiments.

CTTT acquisition in GTs was marked by stable and high-level performance. GTs and STs did not differ in the number of days to reach the performance criterion applied to training stages (70% cued responses to both cues for two consecutive days). Animals needed to reach this criterion to advance through task-training stages to microelectrode array (MEA) implantation and experimental testing (days: $F_{(1,43)} = 0.04$, $p = 0.84$, Fig. 2.6b). As described in the methods, cohorts were trained separately over 19 months (see Methods for details on cohorts). No effect of cohort, modality, or sex was found on days to reach criteria ($F < 1.01$, $p > 0.32$, for all factors).

Baseline CTTT performance across phenotypes. Upon reaching CTTT performance criteria and prior to further manipulation, four consecutive test sessions were videotaped to determine baseline performance. Performance stability was calculated as the standard deviation of stop and turn scores for each rat across the four sessions. STs displayed greater variability than GTs (phenotype: $F_{(1,42)} = 16.19$, $p < 0.001$, $\eta_p^2 = 0.28$, Fig. 2.6c). Although there was greater variability in the performance of STs, all animals demonstrated comparable learning rates. This suggests that rats of different phenotypes learned the CTTT.

Performance measures (turn and stop scores) were analyzed to determine potential cohort-specific performance levels and stability differences. Turn scores were calculated as the number of turns over the total number of trials where the turn cue was presented.

Stop scores were calculated as the number of stops over the total stop trials. Turn cues triggered relatively more successful turns in GTs than in STs (phenotype: $F_{(1,41)} = 15.94$, $p < 0.001$, $\eta_p^2 = 0.18$, Fig. 2.6d). Stop scores did not differ significantly across GTs and STs (phenotype: $F_{(1,41)} = 0.46$, $p = 0.50$, Fig. 2.6e).

Selection of trials used for the analysis of glutamate concentrations. The first day of amperometric recordings was used for these analyses as the rodents used here were exposed to different manipulations (described in detail in *Chapters III* and *IV*). Extracellular glutamatergic (GLU) concentrations, time-locked to the onset of turn and stop cues, were compared across response categories: cued turns, missed turns, cued stops, and false turns. Cued turns were defined as trials where the turn cue was presented, and animals turned before the treadmill belt restarted. Conversely, failing to turn before the treadmill restarted was counted as a missed turn. Cued stops were defined as trials where the stop cue was presented, and animals stopped and did not turn. A stop cue-triggered turn prior to the restarting of the treadmill was classified as a false turn. GLU concentrations during banana pellet delivery resulting from cued turns and stops were also compared across GTs and STs.

Analyses of extracellular GLU concentrations: Overall strategy. Given the complexity of the experimental design (effects of phenotype on turn and stop rates elicited by turn and stop cues of either modality), here we first determine potential differences in GLU levels across trial types and response categories within the individual phenotypes. Based on the main findings from these analyses, contrasts between the GTs and STs will be determined further below. Findings from INs were not included as their glutamate levels were generally highly variable, mirroring in part cue-locked increases seen in GTs and in part in STs.

GLU concentrations in GTs. To account for repeated measures, linear mixed-effects models (LMMM) were used to test differences between GLU dynamics (amplitude, time to peak, and number of peaks) in GTs during cued turns, missed turns, cued stops, and false turns. Cue-locked GLU amplitudes during cued and false turns were significantly larger than during missed turns and cued stops (response: $F_{(3,288.43)} = 64.95$, $p < 0.001$, $\eta_p^2 = 0.40$, Fig. 2.7a). These increases peaked sooner during cued turns, approximately

1 s relative to cue onset, compared to missed turns ($F_{(3,289.56)} = 4.66$, $p = 0.003$, $\eta_p^2 = 0.05$, Fig. 2.7b). Additionally, increases in GLU levels during cued stops and missed turns in GTs displayed more peaks compared to cued turns (response: $F_{(3,288.54)} = 7.83$, $p < 0.001$, $\eta_p^2 = 0.06$, Fig. 2.7c). To sum these findings, transient GLU concentration changes rapidly peaked to a greater degree during detected turn cues than in trials where the cue was missed or during stop cue trials. This pattern suggests the possible involvement of glutamate in the dorsomedial striatum during cue-guided scenarios, and play a role in adaptive movement responses in goal-tracking rats.

Trial- and outcome-independent glutamate concentrations in STs. linear mixed-effects models (LMMMs) were employed to examine the differences between GLU dynamics in STs across different responses as done in GTs. The LMMM revealed that the amplitudes of cue-locked GLU increases in STs during cued turns, missed turns, cued stops, and false turns were not significantly different (response: $F_{(3,243.09)} = 2.60$, $p = 0.053$, Fig. 2.7d). These increases during cued stops occurred closer to the cue onset when compared cue-locked increases during cued and missed turns (response: $F_{(3,242.75)} = 5.78$, $p < 0.001$, $\eta_p^2 = 0.06$, Fig. 2.7e). Additionally, there were significantly fewer peaks in STs during cued stops than in cued turns (phenotype: $F_{(3,243.05)} = 8.71$, $p < 0.001$, $\eta_p^2 = 0.08$, Fig. 2.7f). In sum, increases in transient GLU concentrations were indistinguishable in amplitude across responses in STs. However, cue-locked GLU increases during cued stops rapidly peaked compared to turn trials. This pattern indicates that dorsomedial striatal glutamate might not be central to guiding STs' performance in the CTTT.

GLU levels across phenotypes. Using linear mixed-effects models (LMMs) to account for repeated measures and unequal group sizes, we compared GLU dynamics between GTs and STs across response categories and during reward delivery. During cued turns, amplitudes of GLU increases in GTs were significantly larger compared to STs (phenotype: $F_{(1,184)} = 9.27$, $p = 0.003$, $\eta_p^2 = 0.05$, Fig. 2.8b). Moreover, in STs, these cue-locked increases peaked later, than in GTs (phenotype: $F_{(1,27.07)} = 17.94$, $p < 0.004$, $\eta_p^2 = 0.40$, Fig. 2.8c). Furthermore, STs exhibited a significantly higher number of peaks than GTs (phenotype: $F_{(1,26.87)} = 8.46$, $p = 0.007$, $\eta_p^2 = 0.24$, Fig. 2.8d). During missed turns, GLU increases in GTs were significantly lower than in STs (phenotype: $F_{(1,12.19)} = 28.28$, $p < 0.001$, $\eta_p^2 = 0.70$, not shown). Additionally, GLU increases time-locked to the stop cue

in STs were larger compared to GTs (phenotype: $F_{(1,25.15)} = 22.21$, $p < 0.001$, $\eta_p^2 = 0.47$, Fig. 2.9b). Furthermore, these increases displayed more peaks in STs than in GTs (phenotype: $F_{(1,29.48)} = 9.53$, $p = 0.004$, $\eta_p^2 = 0.24$, Fig. 2.9d). No significant differences in GLU concentration levels were observed between phenotypes during false turns (phenotype: $F < 2.32$, $p > 0.14$, for all measurements, not shown).

Given how infrequently animals missed turn trials and falsely turned to stops, only data collected during cued turns and stops were used in the later chapters. As animals were rewarded banana pellets when successfully performing cued turns and stops, GLU concentrations were time-locked to reward delivery and compared across phenotypes. During banana pellet delivery awarded for cued turns, the amplitudes of GLU increases in GTs were significantly lower than in STs ($F_{(1,16.01)} = 11.09$, $p = 0.004$, $\eta_p^2 = 0.41$, Fig. 2.10a). Reward delivery resulting from cued stops showed the same pattern with significantly lower GLU amplitudes in GTs than in STs ($F_{(1,22.80)} = 12.22$, $p = 0.002$, $\eta_p^2 = 0.32$, Fig. 2.10b).

Conclusions. Collectively, these findings indicate distinct levels of dorsomedial striatal GLU across phenotypes. While, in GTs, cues that triggered turns evoked robustly higher GLU levels than cues yielding missed turns, in STs, GLU levels did not differ between these trial types. Conversely, the reward delivery evoked higher GLU levels in STs than in GTs. As will be discussed further below, and also in the context of effects of inhibition of cortico-striatal projections on GLU levels and turning performance (Chapter IV), these findings may reflect that cued turning in GTs involves a top-down, cortico-striatal integration of cue information into ongoing movement sequences. In contrast, cued turning in STs does not depend on such a mechanism but utilizes perhaps largely entirely striatal processes to execute well-practiced turns; GLU during reward delivery appears to be elevated in STs, which may support this interpretation.

Table 2.1 Acquisition stages of the cue-triggered turning task

day	initial speed	final speed	speed increased
0	habituation to treadmill, motor turned off		
1	3.2 cm/s	8.0 cm/s	3 times by 1.6 cm/s, every 2 min
2	3.2 cm/s	8.0 cm/s	
3	6.2 cm/s	9.6 cm/s	2 times by 1.6 cm/s, every 5 min
4	6.2 cm/s	9.6 cm/s	
5	8.0 cm/s	9.6 cm/s	once, after 10 min
6	8.0 cm/s	9.6 cm/s	once, after 10 min
7	9.6 cm/s		n/a
day	speed	trials	
8-19	9.6 cm/s	18 turn and stop cues/session	

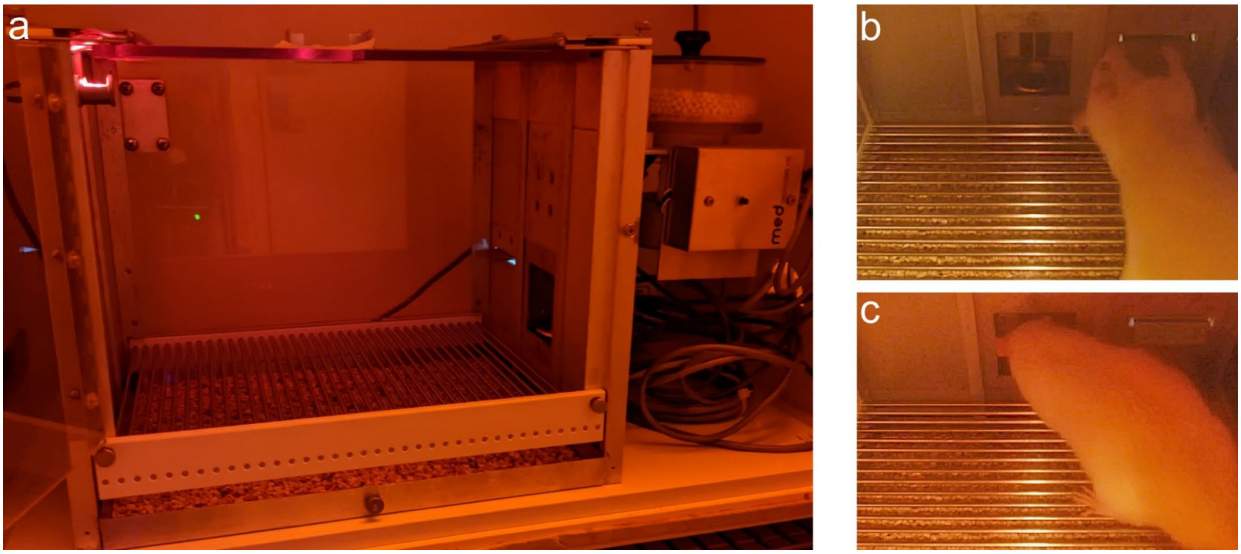


Figure 2.1: Pavlovian conditioned approach chamber and index scores
Representation of the operant chamber (a) equipped with a goal cup, pellet dispenser, red house light, illuminated lever, and metal grate flooring used in Pavlovian Conditioned Approach (PCA) screening. Five consecutive PCA sessions were used to screen and assign phenotype. Illumination of a red house light (shown on the top left) indicated the start of a session comprised of 25 trials. The illuminated lever (shown left to the goal cup) extended into the chamber for a duration of 8s. Immediately after lever retractiong, a pellet was delivered into the goal cup. 25 lever-pellet pairings were presented on a variable Interval-90 (VI-90) schedule, with each session lasting approximately 37.5 minutes. Averaged values from days 4 and 5 generated index scores. The cutoff range for sign-trackers was scores of 0.5 to 1.0, biasing toward lever-directed behavior (b).The cutoff range for goal-trackers was scores of -0.5 to -1.0, favoring goal cup-directed behavior (c). Animals with scores between these two ranges were classified as intermediates.

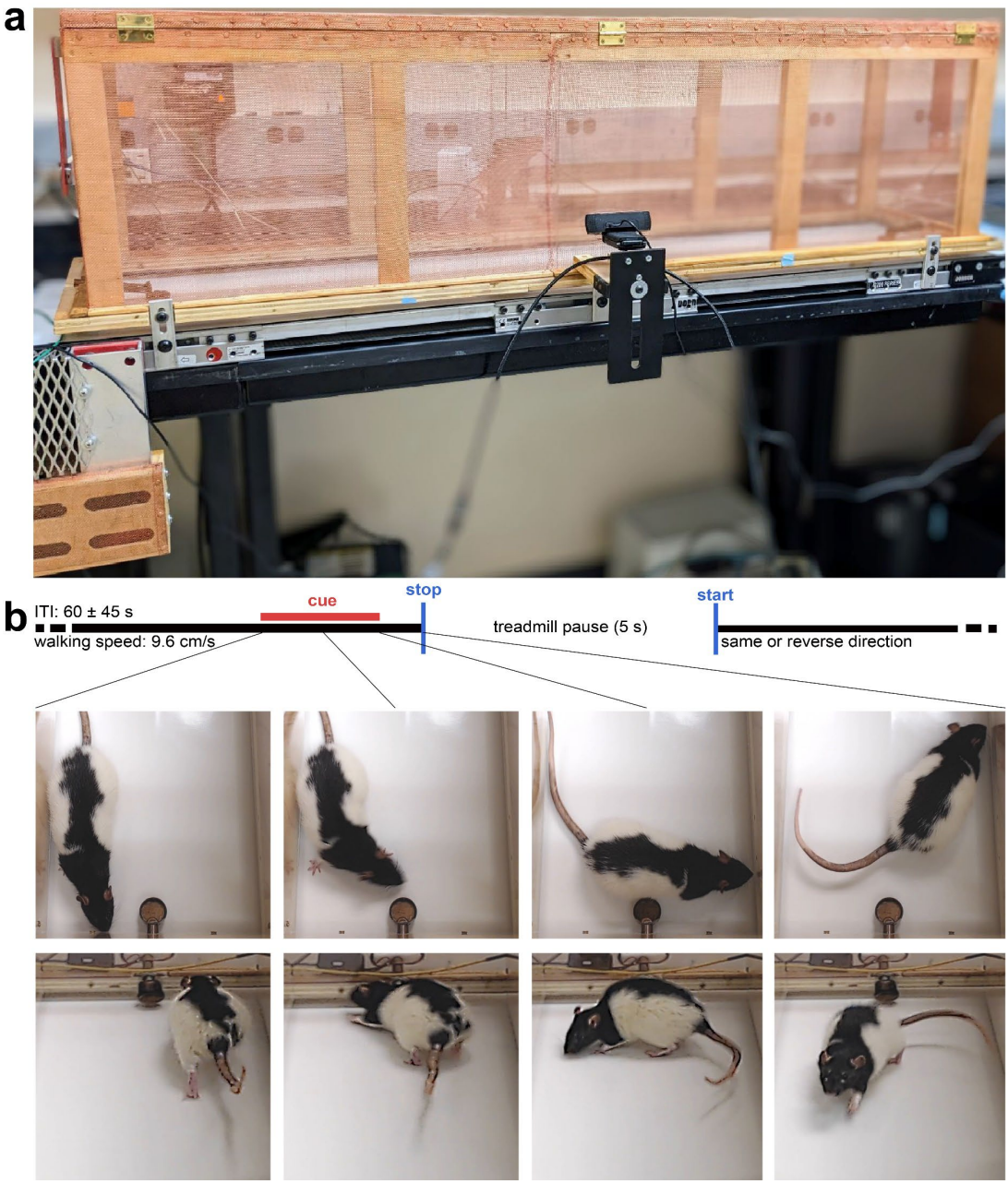


Figure 2.2: Cue-triggered turning apparatus and schematic

a: Illustration of the 1.5-m long CTTT, with Faraday shielding comprised of copper wire mesh surrounding the motor and wooden frame of the Plexiglas-lined enclosure (both grounded), 2 mounted cue lights on both ends, 1 mounted audible device centered (not visible here), two copper reward ports on either end and two wooden panels on top of the cage to place rodents. Four web cameras were placed on all sides of the treadmill. b: The CTTT required rats to walk on a treadmill (walking speed: 9.6 cm/s) and to utilize the presentation of a turn cue (tone or light for 2 s), indicating that the treadmill will stop and restart 6 s later, in the reverse direction. The other cue (tone or light) predicted a treadmill restart in the same direction (correct trials were rewarded with a 45 mg banana pellet). The photographs show a rat initiating the turn within 500 ms of the cue onset (see lines connecting the photos to the task timeline and completing this turn by the time the treadmill stopped. Cues were separated by a variable intertrial interval (ITI) of 60±30 s during which the rat continued walking the treadmill.

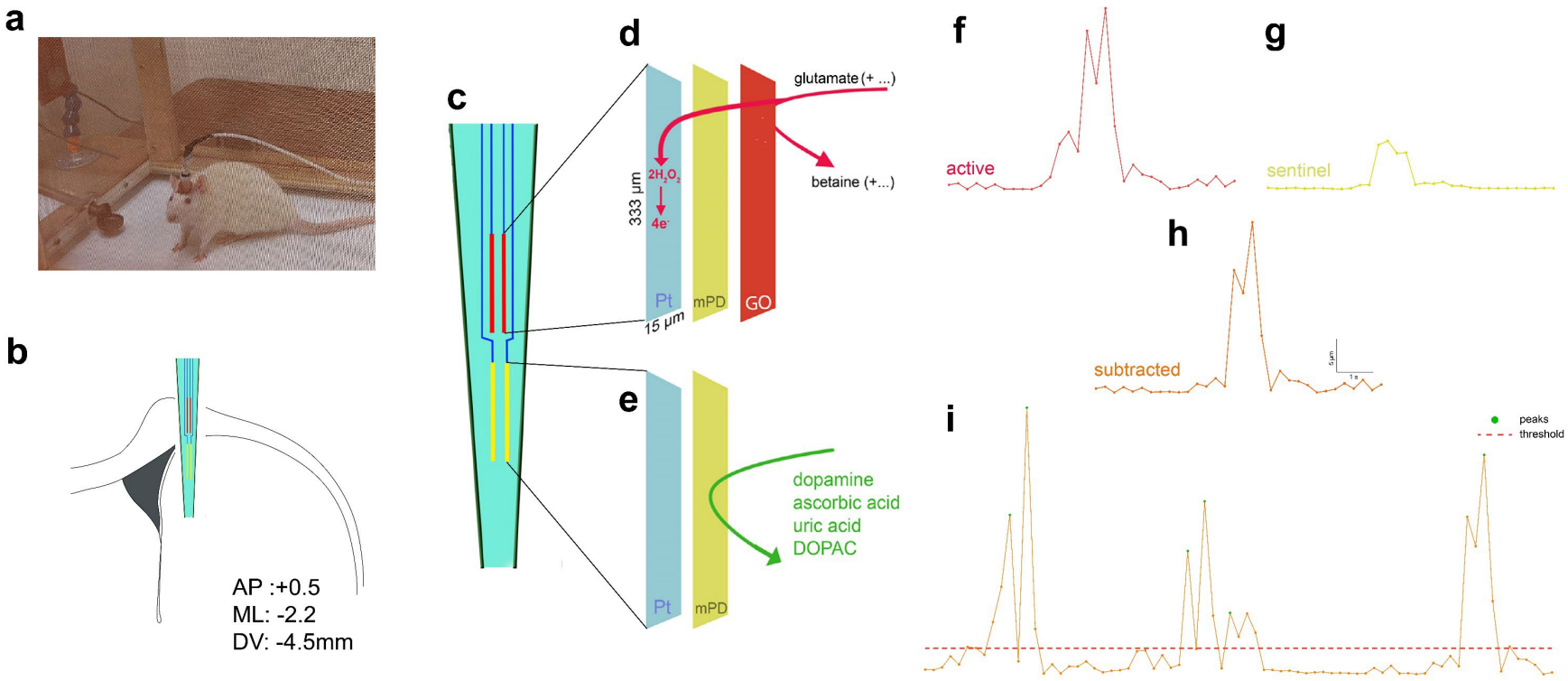


Figure 2.3: Amperometry data analysis
a: Rat with a microelectrode array (MEA) head stage connected to a FAST-16 potentiostat/data electrochemical system via a shielded cable and low-impedance commutator. b: MEAs were inserted into the right dorsomedial striatum (coordinates shown in mm from Bregma). c: Ceramic backbone with four platinum-iridium (Pt/Ir) recording sites, organized in two pairs, with each site measuring 333 µm long and 15 µm wide. The upper pair measured glutamate currents (red, also d), while the lower pair served as sentinels for background subtraction (yellow, also e). Glutamate oxidase (GO) was immobilized onto the upper pair of recording sites. Application of a 0.7 V potential versus the Ag/AgCl reference electrode (not shown) during recordings oxidized glutamate. After GO coating, a non-conducting polymer, m-(1,3)-phenylenediamine (mPD), was electroplated onto the sites to block the transfer of small electroactive organic molecules like dopamine to the Pt sites. However, the mPD layer did not block hydrogen peroxide, the by-product, from reaching the Pt surface. The resulting current from the sentinel sites represented background noise and was subtracted from the active sites (g) to calculate the subtracted current (h). Example traces with peaks (green circles) detected by the PeakDet Matlab tool. The red dotted line indicates the threshold for peak consideration. Values with a minimum difference were identified as peaks, while those falling below this criteria were not classified as peaks (rightmost trace with one peak, see Methods for criteria selection).

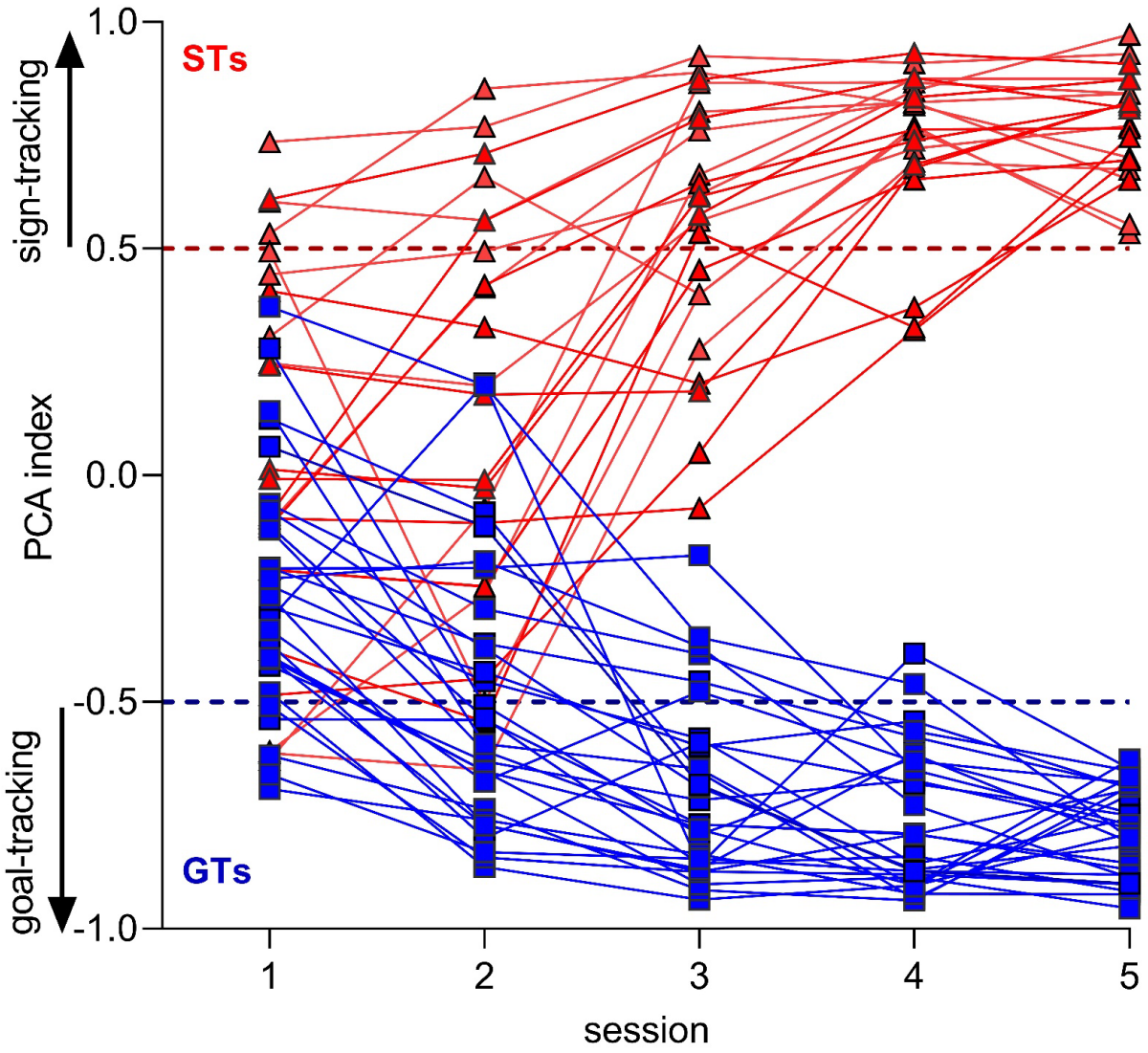


Figure 2.4: Distribution of goal-tracking and sign-tracking rats used throughout experiments.

The phenotypes of goal-tracking rats (blue squares) and sign-tracking rats (red triangles) were assessed across 5 Pavlovian Conditioned Approach sessions (x-axis). Individual PCA index scores averaged from sessions 4 and 5 were used to assign phenotype with predetermined cutoffs of 0.5 (red dotted line) for STs and -0.5 (blue dotted line) for GTs on the y-axis.

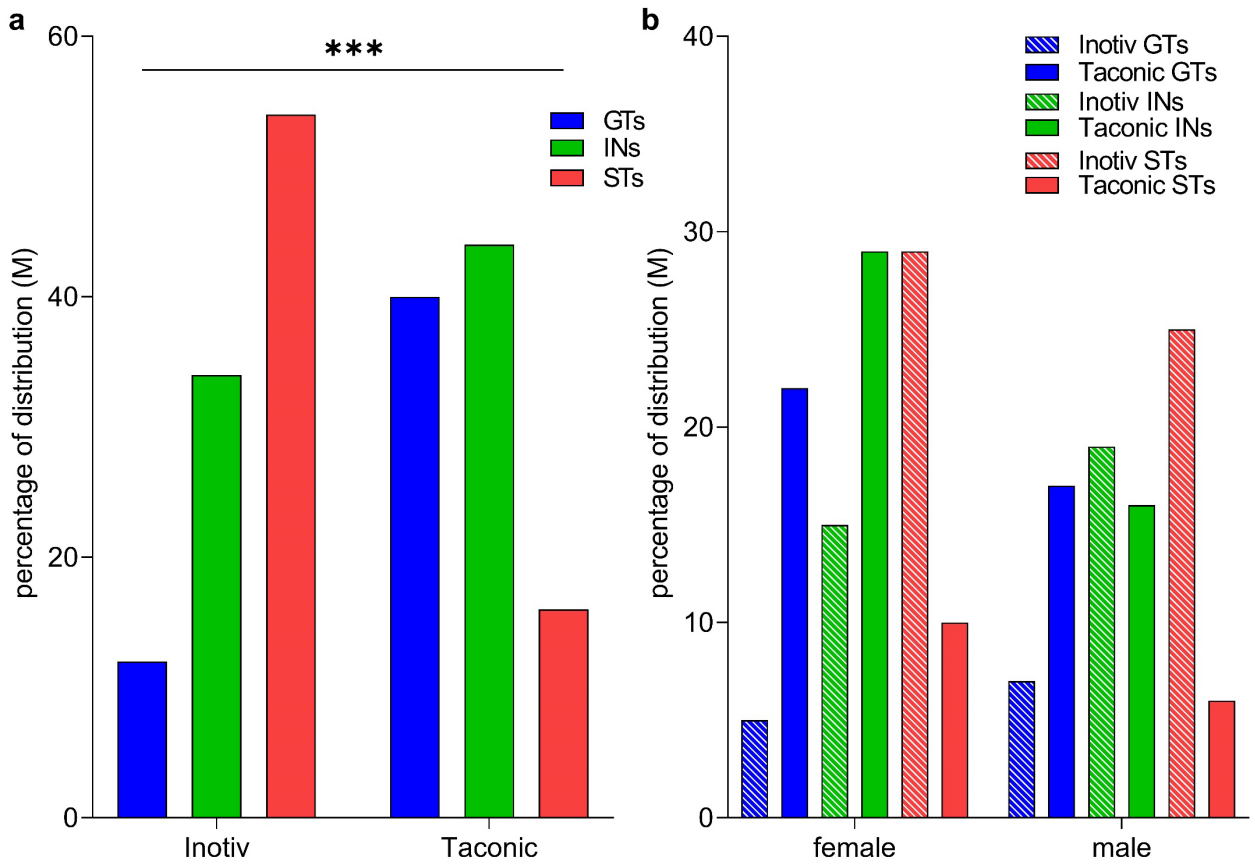


Figure 2.5: PCA distribution across vendors

a: Percentage of goal-tracking (blue), intermediate (green), and sign-tracking (red) rats between Inotiv and Taconic cohorts. ***above single bar indicates a main effect of (p<0.001) of vendor. b: Percentage distribution of each phenotype by sex for Inotiv (bars with diagonal white lines) and Taconic (solid bars). No significant differences were found in the phenotype distribution based on sex within either vendor.

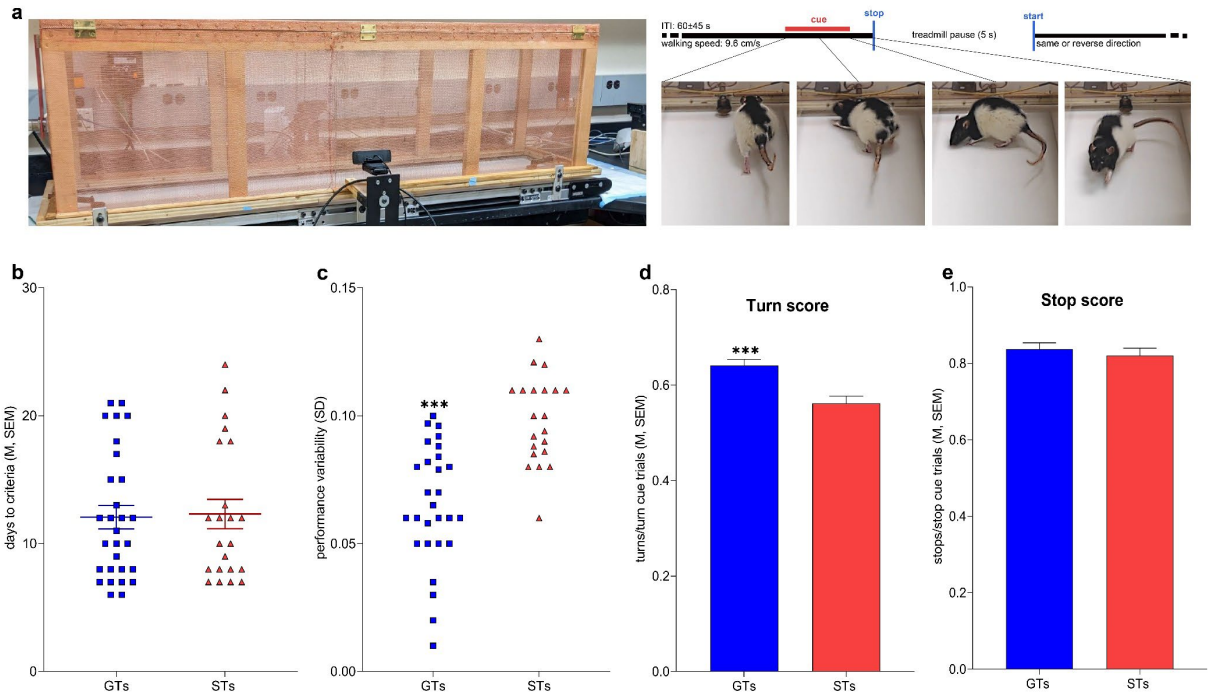


Figure 2.6: CTTT acquisition in STs is variable

a: Cue-triggered turning task (CTTT) apparatus equipped with four web cameras on each side of the Faraday enclosure; four sessions of cue-triggered turning task sessions were recorded upon animals reaching criteria (used for c-e). b: GTs (blue squares) and STs (red triangles) had similar learning rates (days to criteria, represented as M±SEM) for the CTTT. c: GTs exhibited lower performance variability (standard deviation) compared to STs. d: GTs (blue bars) displayed a higher rate of cued turns (represented as EMM±SEM) compared to STs (red bars). e: No differences in the number of stops per stop cue trials between GTs and STs were found. ***above GTs indicates a main effect (p<0.001) of phenotype.

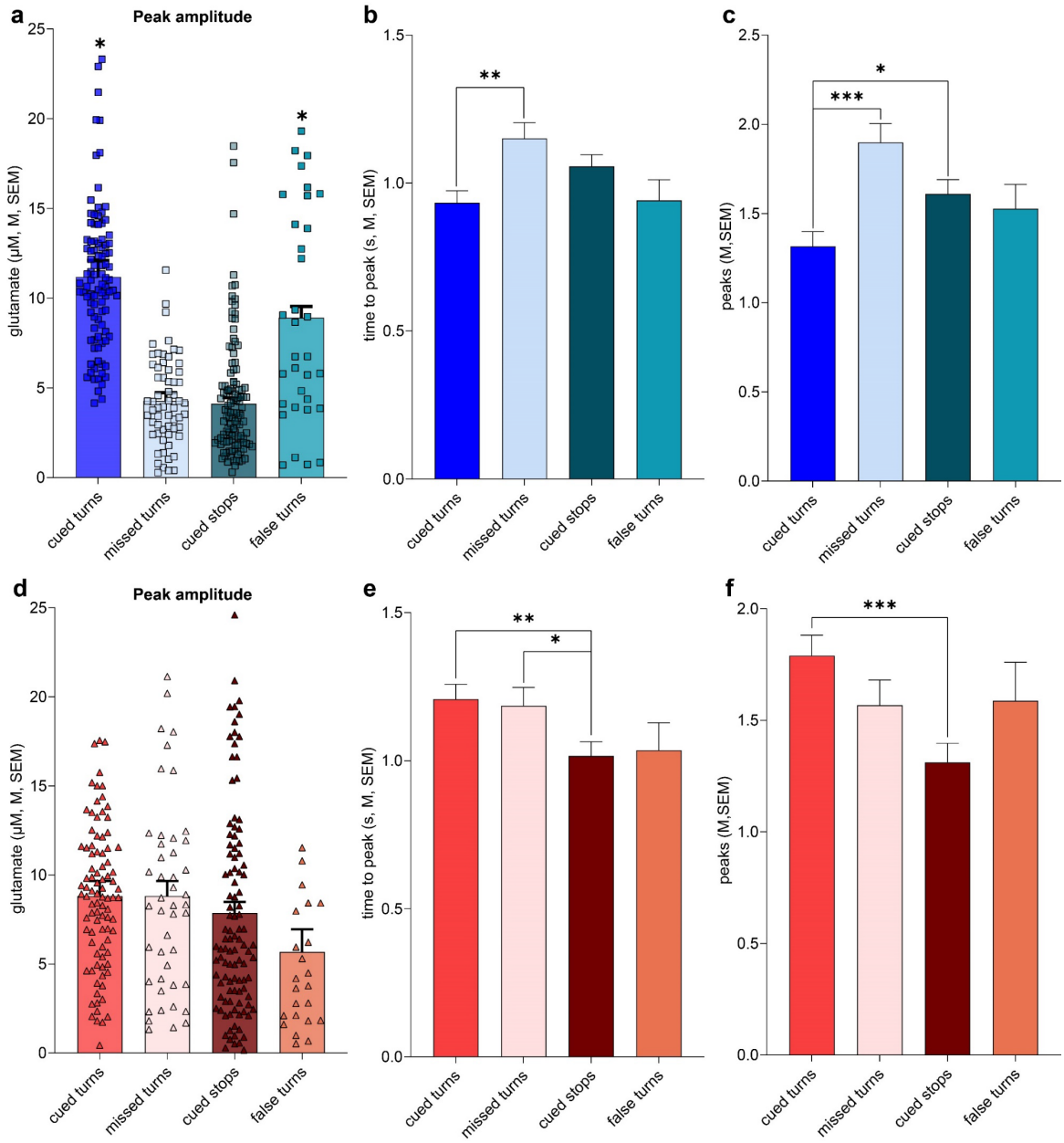


Figure 2.7: GLU dynamics across responses in GTs and STs

a: Differences in dorsomedial glutamate (GLU) concentrations (represented as EMM±SEM with individual values superimposed) time-locked to task cues in GTs during cued turns (blue), missed turns (light blue), cued stops (dark blue), and false turns (teal). Glutamate concentration in µM displayed on the y-axis. GTs showed larger transient GLU increases during cued and false turns compared to missed turns and cued stops. b: GLU in GTs increased faster during cued turns than missed turns. c: GTs exhibited fewer peaks during cued turns than missed turns and cued stops. d: Amplitude differences om GLU concentrations time-locked to task cues in STs during cued turns (red), missed turns (pink), cued stops (dark red), and false turns (orange). Amplitudes in STs were not significantly different between responses. e: During turns, GLU increases were slower to peak than in cued stops and peaked less often (f). Post-hoc comparisons between responses: $p<0.05$*, $p<0.01$**, $p<0.001$***.

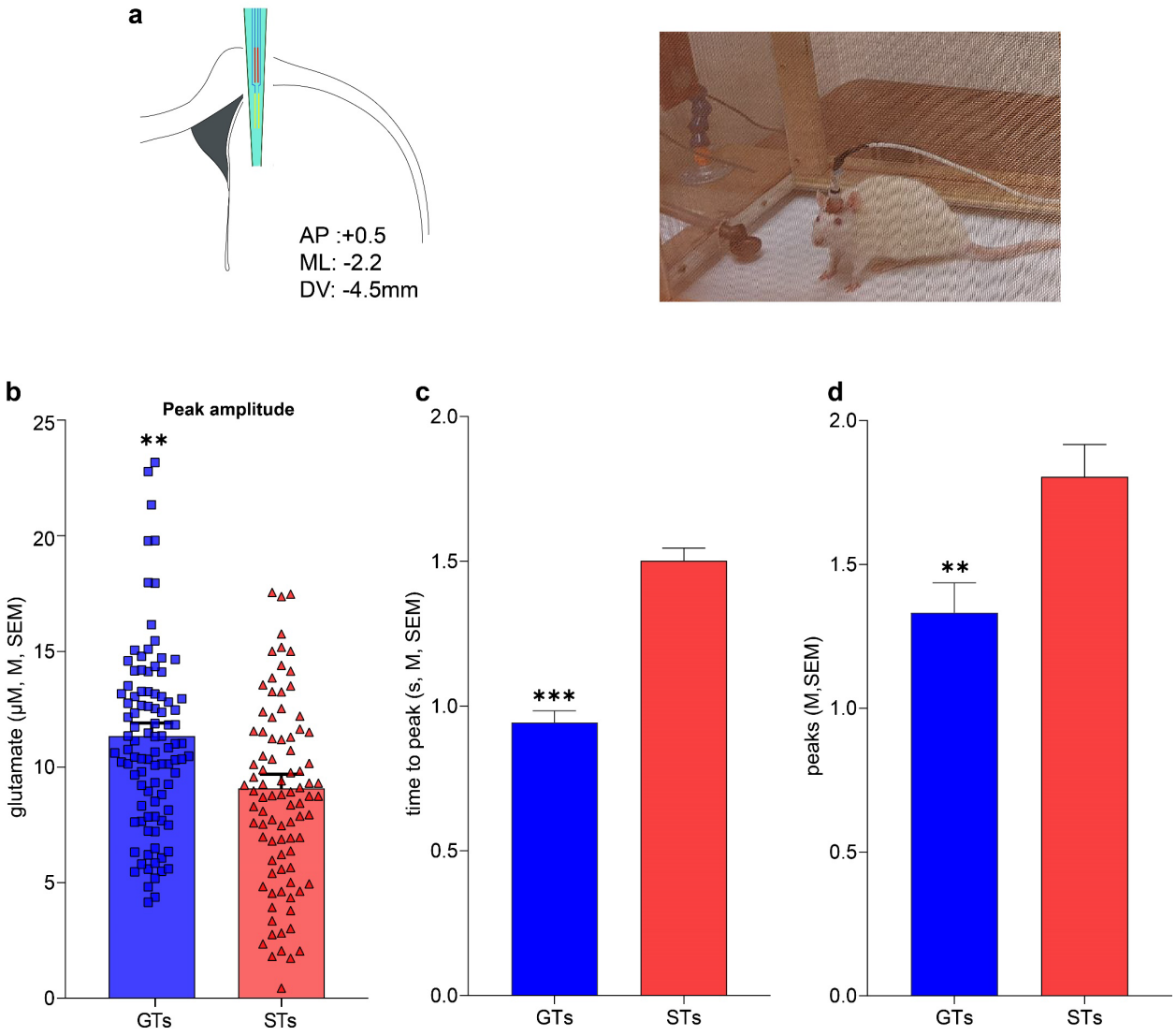


Figure 2.8: Elevated GLU increases in GTs compared to STs during cued turns
Differences in dorsomedial glutamate (GLU) dynamics across GTs and STs during cued turns. a: Placement of amperometric glutamate-sensitive biosensor in the DMS for *in vivo* recordings in GTs and STs performing the CTTT. b: Amplitudes of glutamate (GLU) concentrations (represented as EMM±SEM with individual values superimposed) time-locked to the turn cue in GTs (blue) and STs (red). Transient GLU increases in GTs during cued turns were significantly larger compared to STs. **above GTs indicates a main effect (p<0.01) of phenotype. Glutamate concentration in µM displayed on the y-axis. c: GLU concentrations in STs during turns increased slower (in seconds) than in GTs. ***above GTs indicates a main effect (p<0.001) of phenotype. d: There were fewer peaks in GTs than in STs. **above GTs indicates a main effect (p<0.01) of phenotype.

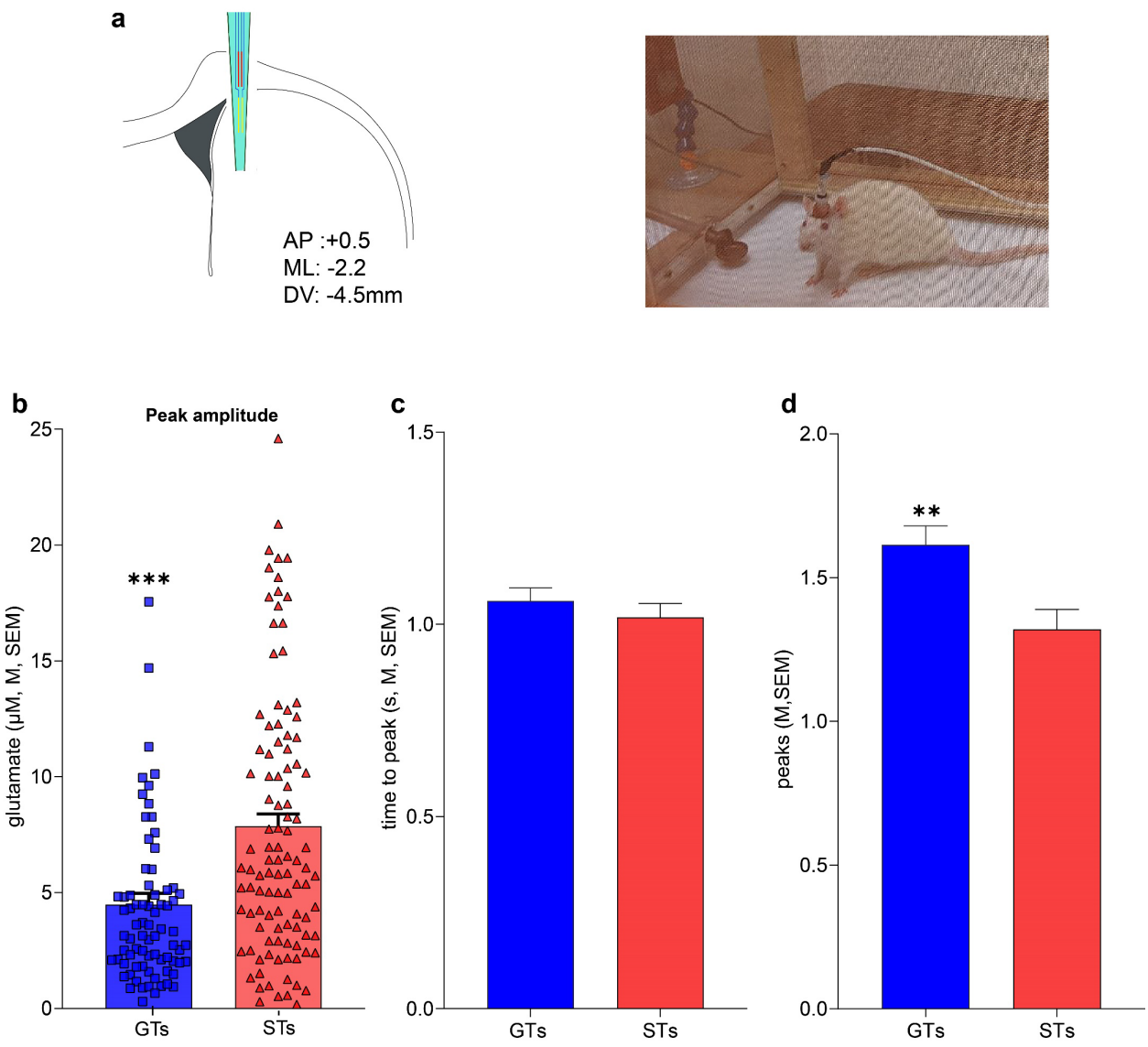


Figure 2.9: Differences in GLU dynamics between STs and GTs during cued stops
a: Placement of amperometric glutamate-sensitive biosensor in the DMS for *in vivo* recordings in GTs and STs performing the CTTT. b: Amplitudes of dorsomedial glutamate (GLU) concentrations (represented as EMM±SEM with individual values superimposed) time-locked to the stop cue in GTs (blue) and STs (red) during cued stops. GLU increases in GTs during cued stops were significantly smaller compared to STs. ***above GTs indicates a main effect (p<0.001) of phenotype. Glutamate concentration in µM displayed on the y-axis. c: Transient GLU increases in GTs and STs during stops peaked around the same time (in seconds). d: There were more peaks in GTs than in STs during cued stops. **above GTs indicates a main effect (p<0.01) of phenotype.

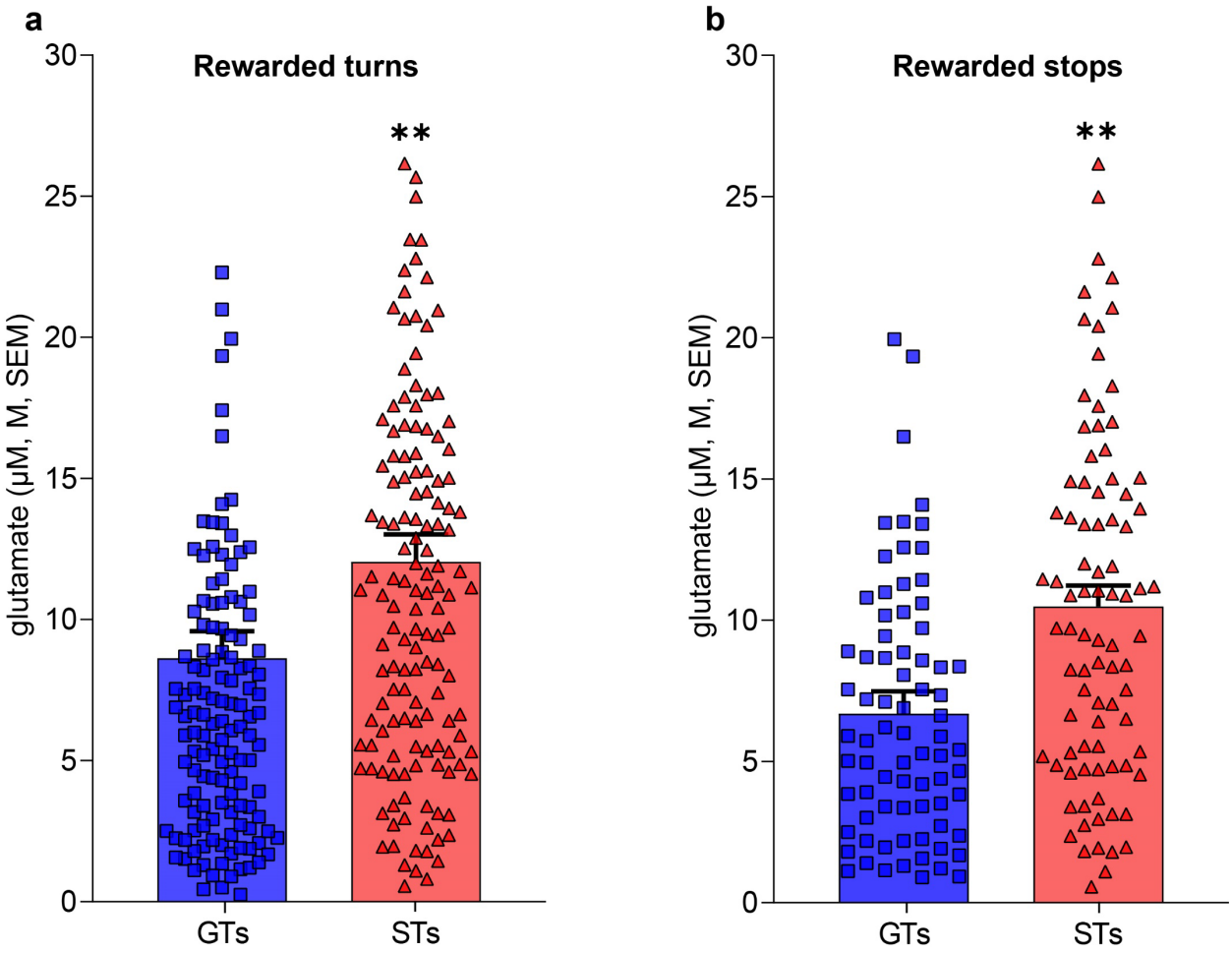


Figure 2.10: Elevated GLU increases in STs during reward delivery
a: Amplitudes of dorsomedial glutamate (GLU) concentrations (represented as EMM±SEM with individual values superimposed) time-locked to reward-delivery in STs (red) were significantly larger than in GTs (blue) for rewarded (cued) turns. **above GTs indicates a main effect (p<0.01) of phenotype. Glutamate concentration in µM displayed on the y-axis. b: GLU concentration increases in STs at reward delivered were significantly larger compared to GTs for rewarded (cued) stops. **above GTs indicates a main effect (p<0.01) of phenotype.

Chapter III: The Role of Corticostriatal GLU in GTs and STs for CTTT Performance

Abstract

Navigating dynamic and challenging environments requires effective top-down attentional regulation facilitated by attentional shifts. We hypothesize that these shifts aid in cortical cholinergically mediated cue detection are transferred via glutamatergic (GLU) projections to integrate with striatal movement sequencing, guiding movements, and corrective actions. To investigate this hypothesis, we conducted dorsomedial striatal recordings via glutamate-sensitive biosensors in animals with opposing attentional-motivational styles, sign-trackers (STs), and goal-trackers (GTs) in a cue-triggered turning task with modified parameters. We manipulated the inter-trial interval (ITI) and varied trial-type proportions to challenge performance in GTs and examined associated GLU concentrations. STs are proposed to rely on bottom-up attentional processing, and, therefore, may be insensitive to demands on top-down processing. Shortening the ITI increased GLU amplitudes in STs without influencing turning. Unexpectedly, none of these manipulations affected cue-triggered turning or GLU concentrations in GTs. Shortening the ITI reduced GLU amplitudes without influencing turning whereas decreasing the number of turn trials decreased cue-triggered turning without affecting dorsomedial GLU concentrations. These findings highlight a potential nuanced involvement of corticostriatal glutamatergic projections in cue detection. Existing evidence suggests that these projections may play a role in updating action-outcome associations, temporal cue processing, or transitioning between alternatives, and our results underscore a more intricate relationship. Further investigation within individuals and future research endeavors will be essential to unveil the specific contribution of

corticostriatal glutamate in these processes, ultimately influencing and driving goal-directed actions.

Introduction

Exiting the grocery store, you stroll towards the parking lot, only to encounter a common dilemma: where did you park? You can employ different strategies in your search. Approaches reliant on the perceptual features of your car, such as the blue paint, may be rendered ineffectual due to vehicles of similar hues, interfering with this “bottom-up” approach. Consequently, you mentally backtrack, recalling that you parked in the western section of the lot near a cart corral. You decide to limit approaches to blue sedans within proximity of cart return areas and a specific spatial boundary. Such a targeted search employs “top-down” attentional mechanisms to facilitate cue detection. We propose that effective navigation in dynamic and challenging environments necessitates top-down attentional regulation, achieved through attentional shifts. These shifts aid cortical cholinergically mediated cue detection, transferred via glutamatergic (GLU) projections to integrate with striatal movement sequencing to guide movements and corrective actions. To test this hypothesis, we recorded animals of opposing attentional-motivational styles, sign-trackers (STs), and goal-trackers (GTs).

Top-down attentional regulation is classified as knowledge-driven, goal-driven, and voluntary (Kastner & Ungerleider, 2000; Sarter et al., 2001). Top-down mechanisms, including heightened sensory processing, switching between decision criteria, directing attention to specific cue locations, and filtering out distractions, aid in cue detection. Here, 'detection' refers to a psychological process that amplifies cue processing, evoking responses based on pre-established stimulus-action learning (Posner, 1980). In contrast, bottom-up attention relies on a target cue's sensory or perceptual salience to trigger cortical region recruitment (Treisman & Gelade, 1980). Cognitive-motivational styles - preexisting attentional and motivational biases - can significantly shape an individual's criteria for determining which cues trigger behavior (Pitchers et al., 2017a; Sarter & Phillips, 2018).

STs exhibit “hot” cognitive-motivational styles defined as biasing towards striatal and prefrontal dopaminergic processing of the motivational features of cues (Flagel et al., 2011; Gillis & Morrison, 2019; Paolone et al., 2013; Pitchers et al., 2017a). This cognitive-

motivational style is evident in a tendency to attribute incentive salience to cues temporally associated with unconditioned stimuli such as rewards. STs heavily depend on predictive cues with cue presentation invigorating instrumental actions (Robinson et al., 2014). The bottom-up biases observed in STs manifest as increased performance instability leading to heightened susceptibility to attentional distractors and greater variability within sessions compared to GTs (Paolone et al., 2013; Sarter & Phillips, 2018). These attentional deficits are linked to significantly lower task-induced cortical cholinergic signaling in STs than GTs. This reduced signaling capacity is enforced by unresponsive choline transporters, rate-limiting factors in the synthesis of acetylcholine (Koshy Cherian et al., 2017; Sherman et al., 1978). Conversely, GTs display “cold” cognitive-motivational styles defined as analytical cortical cholinergic processing of the utility of cues. This style becomes evident during tasks involving Pavlovian cues, where GTs use these cues as predictors of reward availability (Flagel et al., 2011; Meyer et al., 2012). Correspondingly, prefrontal dopamine levels in GTs do not increase during these tasks, as observed in STs (Pitchers et al., 2017a). Contextual cues that signal learned action-outcome associations significantly impact behavior in GTs (Colaizzi et al., 2020; Pitchers et al., 2017b; Pohořalá et al., 2021; Robinson et al., 2014). For instance, "occasion-setters" drive drug-seeking behaviors in these animals, reflecting an expectation of the drug and heightened motivation. Moreover, cholinergic lesioning diminishes the ability of occasion-setting cues to drive these behaviors. Furthermore, in GTs, inhibiting basal forebrain-cortical inputs results in vigilance decrements, inefficient attentional supervision of motor cues, and increased susceptibility to distractors (Kucinski et al., 2022; Kucinski et al., 2019).

Collectively, these findings suggest that in attentionally taxing circumstances, GTs bias toward “top-down” attentional processing regulated by the cortical cholinergic system. On the other hand, STs may adopt alternative strategies biased towards "bottom-up" attentional processes, thereby not necessitating intact cortical cholinergic signaling (Phillips & Sarter, 2020). While bottom-up and top-down processes often work in concert and should not be considered dichotomous systems, studying these animal models that exhibit a predisposition toward either provides a valuable opportunity to dissect the underlying neural circuitry and cognitive processes responsible for cue-guided complex

movement (Awh et al., 2012; Buschman & Miller, 2007; McMains & Kastner, 2011; Sarter et al., 2001).

Here, we conducted amperometric recordings in the dorsomedial striatum of GTs and STs, performing a cue-triggered turning task with modified parameters. *Chapter II* emphasized the potential roles of dorsomedial glutamate (GLU) in distinct cognitive biases/processes across GTs and STs. In GTs, GLU appeared to contribute to cue-driven attentional processing, aligning with the notion that GTs rely on top-down cortical integration of cues for ongoing goal-directed behavior. Conversely, GLU in STs seemed linked to reward-related processes, reflecting the bottom-up saliency of cues and reward outcomes. We altered inter-trial intervals (ITI) and varied the proportions of trial types to challenge the performance of GTs and examine associated glutamate (GLU) changes. By shortening the ITI, we aimed to increase the demands on top-down attentional mechanisms, specifically cue detection and cross-modal discrimination between turn and stop cues. We predicted that GTs would display elevated GLU levels in response to these manipulations, reflecting reliance on cortical detection and striatal integration of cues for performance. In GTs, a shortened ITI (fast event rate) was expected to increase GLU amplitudes due to sustained attentional engagement for consecutive detection of turn cues. Conversely, lengthening the ITI (slow event rate) in GTs was expected to increase GLU amplitudes and enhance cue-triggered turning. Past work highlighted distractibility and variable performance in sustained attention of STs (Paolone et al., 2013). Therefore, the slow event rate may cause animals to disengage from the task and promote distractibility that STs reflected in lower cue-triggered turns. We predicted that a fast event rate would not affect STs' performance. As shown in *Chapter II*, amplitudes during turn presentation do not seem to drive STs' performance; thus, we did not predict any impacts on GLU amplitudes during this period. We hypothesized that GTs depend on turn cues to perform the CTTT; therefore, we predicted varying the ratio of turn cue presentations would have a corresponding effect on the number of turns exhibited by these animals. In GTs, reducing turn cue presentations (20%) was predicted to decrease cue-triggered turning, while increasing presentations (80%) would lead to more cued turns.

We predicted these manipulations thought to tax top-down control would be ineffective in STs due to their reliance on bottom-up attentional processing. However, there is

substantial evidence that shortening ITIs increases goal-tracking behavior, whereas extending ITIs increases sign-tracking behavior (Cinotti et al., 2019; Mahmoudi et al., 2023). We found that shortening the ITI increased GLU amplitudes in STs without influencing cue-triggered turning. These augmented amplitudes may reflect increases in dopaminergic (DA) modulation as long ITIs are proposed to increase DA activity (Lee et al., 2018). However, none of these manipulations concurrently affected cue-triggered turning or GLU concentrations in GTs. Shortening the ITI attenuated GLU amplitudes without influencing cue-triggered turning. Similarly, reducing the number of turn trials led to decreased cue-triggered turning without affecting dorsomedial GLU concentrations.

Increased demands on cue detection are thought to promote cortical cholinergic transients. We did not observe proportional increases in glutamate levels in dorsomedial striatum to these increased demands, indicating a nuanced role of the information "imported" by corticostriatal glutamatergic projections. There is ample evidence suggesting the projections may be involved in updating action-outcome associations, temporal processing of cues, or transitioning between behavioral alternatives (Chatham et al., 2014; Emmons et al., 2017; Hart et al., 2018; van Schouwenburg et al., 2012). Further analyses within individuals and future research may elucidate if corticostriatal glutamate plays a part in the abovementioned processes to drive goal-directed actions.

Methods

Subjects. 184 Sprague Dawley rats (obtained from Inotiv, West Lafayette, IN and Taconic, Rensselaer, NY; n = 115 females and n = 69 males; 250-500g) were individually housed on a 12-hour light/dark cycle (lights on at 7:00 AM) at ~21ºC with ad libitum access to food (Laboratory Rodent Diet 5001, LabDiet) and water. The experiments detailed below used four cohorts of rats (n = 36, 19 females). All cohorts were mixed between Taconic and Inotiv. All rats in these experiments underwent amperometric recordings; therefore, cohorts were obtained across 17 months due to the limiting factor of a singular recording system. 12 rats (n = 6 females) were used in the varied ratio of trial types experiment, and 24 rats (n = 14 females) were used in the altered intra-trial interval (ITI) experiment. The remaining animals of the 184 total were used for other experiments. All experimental procedures were approved by the University Committee on the Use and Care of Animals (UCUCA) at the University of Michigan and carried out in accordance with the guidelines

of the Association for Assessment and Accreditation of Laboratory Animal Care. Animals acclimated to housing quarters for two days before handling and manipulation.

Experimental timeline. Figure 3.1 illustrates the timeline of experiments described here. Initially, rats underwent habituation to housing facilities and experimenters via handling for six consecutive days. As discussed in *Chapter II,* rats were screened for goal-tracking and sign-tracking phenotypes for another six days. Figure 3.2 shows the distribution of phenotypes used. Cue-triggered turning task training was conducted for approximately 14 days, after which microelectrode arrays were implanted into the right dorsomedial striatum upon passing the criterion. After a 2-day recovery period, amperometric recordings were performed during test sessions alternating between trained conditions and sessions with either the intertrial interval altered or the ratio of turn trials. Brains were subsequently harvested. A Cresyl Violet Nissl stain confirmed MEA placement. Detailed procedures used in Chapter II can be found in that chapter, while the procedures exclusive to these experiments are described below.

Variations of the intertrial interval and the proportion of trial types. Chapter II details that turn cues evoked brief GLU signals in GTs, while all cues elicited GLU transients, albeit with diminished amplitudes, in STs. We hypothesized that glutamatergic signaling might reflect cholinergic modulation of fronto-cortical inter- and output neurons to striatal inputs to inform cue-guided movements. Therefore, we manipulated the frequency of events/trials and the overall proportion of trial type to increase or decrease demand on fronto-cortical circuitry (Bushnell, 1999; McGaughy & Sarter, 1995).

The term 'turn trials' designates trials that start with the presentation of the turn cue, while 'stop trials' refers to trials that begin with the presentation of the stop cue. The event rate was increased by shortening the inter-trial interval (ITI), called the "short ITI." Conversely, extending the ITI, known as the "long ITI," decreased the event rate. These manipulations involved altering the ITI and were referenced accordingly. For the groups with altered ITIs, a 60±30s ITI was used during training, and the rats were tested with either the short ITI (30±15) or the long ITI (90±45). The animals initially trained with an equal distribution of 50% turn trials and 50% stop trials. Subsequently, we increased the proportion of turn trials to 80% and decreased the ratio of stop trials to 20%. Likewise, we employed the opposite manipulation, where the proportion of stop trials was increased to 80%, and the

ratio of turn trials decreased to 20%. We labeled this group of rats as the "varying ratio group" because the manipulations involved changing the ratio of trial types.

The program for the unaltered and altered CTTT versions timed out after 20 min to prevent physical exhaustion of the rats. Therefore, the sessions with the long ITI had a total number of trials of 13-14. The total number of trials per daily session for all other versions was 18. Training conditions were used for the first amperometric recording session after surgery to ensure a consistent reference point for animals before implementing manipulations. Testing alternated between sessions with training conditions and the assigned manipulation.

Statistical analyses. For CTTT performance measures and glutamate dynamics measurements, linear mixed-effects models (LMMM) were used to account for repeated sessions and unequal group sizes and to test the effects of differing ratios of trial types (20% or 80% turn cue presentations versus the 50% during training) or repeated sessions with altered ITIs (shorter or longer than the ITI used during training). Trial type ratios and altered ITIs were used as the repeated variables, and phenotype, modality, and sex were used as the between-subjects variables with a subject identifier random intercept. We employed Bonferroni *post hoc* pairwise comparisons for significant main effects and interactions.

Results

GTs are sensitive to altering the ITI length. ITI length significantly interacted with phenotype (ITI*phenotype: $F_{(2,10.21)} = 10.30$, $p = 0.004$, $\eta_p^2 = 0.32$, Fig. 3.3). *Post hoc* multiple comparisons indicated that lengthening the ITI (slow event rate) significantly increased the rates of cued turns in GTs compared to sessions with the trained ITI and short ITI ($F_{(2,24)} = 8.52$, $p = 0.02$, $\eta_p^2 = 0.42$, Fig. 3.3d). These comparisons also indicated that altering the ITI did not affect cue-triggered turning in STs ($F_{(2,24)} = 0.77$, $p = 0.48$, Fig. 3.3d). Altering the ITI did not affect the rate of cued stops in GTs or STs (ITI*phenotype: $F_{(2,18)} = 0.24$, $p = 0.79$, Fig. 3.3e).

ITI length decreases and increases GLU concentrations in GTs and STs, respectively. Microelectrode arrays (MEAs) were chronically implanted into the dorsomedial striatum of rats to be recorded while animals performed the CTTT. For the altered inter-trial interval

(ITI) recordings, the MEAs had slopes of 9.30 ± 0.15 pA/μm, a selectivity of 150.87:1.00 ± 4.82 (glutamate:AA), limit of detections of 0.32 ± 0.01 μm glutamate, and highly linear responses of 0.99 ± 0.001. Manipulating the ITI resulted in changes to the maximum amplitude of transient GLU increases in a phenotype-dependent manner (ITI*phenotype: ($F_{(2,285.09)}$ = 4.08, p = 0.02, η_p^2 = 0.06). *Post hoc* multiple comparisons indicated that shortening the ITI resulted in relatively smaller increases in GLU concentrations in GTs executing cued turns compared to increases during the trained and extended sessions ($F_{(2,371)}$ = 6.51, p = 0.002, η_p^2 = 0.02, Fig. 3.4d). For STs, turn cue-locked GLU increases were significantly larger in the shortened ITI condition compared to increases with the long ITI condition ($F_{(2,371)}$ = 4.95, p = 0.008, η_p^2 = 0.02, Fig. 3.4d). No effects of altering ITI length were found on other GLU dynamics (number of peaks or time to peak) during cued turns. ITI alteration did not affect the amplitudes or time to peak of transient GLU increases during cued stops ($F_{(2,169.84)}$ = 1.16, p = 0.32). However, a *post hoc* multiple comparison indicated significantly fewer peaks during cued stops in the short and long ITI sessions compared to the trained ITI sessions ($F_{(2,141.28)}$ = 8.26, p < 0.001, η_p^2 = 0.05, not shown). As seen in Chapter II, GLU increases in GTs peaked more than in STs during cued stops ($F_{(1,12.21)}$ = 5.64, p = 0.04, η_p^2 = 0.02, not shown).

These results indicate that manipulating the intertrial interval (ITI) affected dorsomedial GLU concentrations in phenotype-dependent effect manner. Shortening the ITI resulted in smaller GLU increases in GTs during cued turns, which was contrary to our prediction as a faster event rate should increases on frontal cortical cholinergic activity and thereby be reflected in larger GLU increases. Lengthening the ITI increased the number of cued turns in GTs; however, there was no effect on GLU concentrations. Shortening the ITI resulted in larger GLU increases in STs during cued turns without affecting the number of turns. This augmentation in GLU amplitudes may imply the involvement of bottom-up mechanisms processes, such as dopaminergic modulation of glutamate or thalamic engagement, as shortening the ITI increased the reward rate and the event rate (see Discussion).

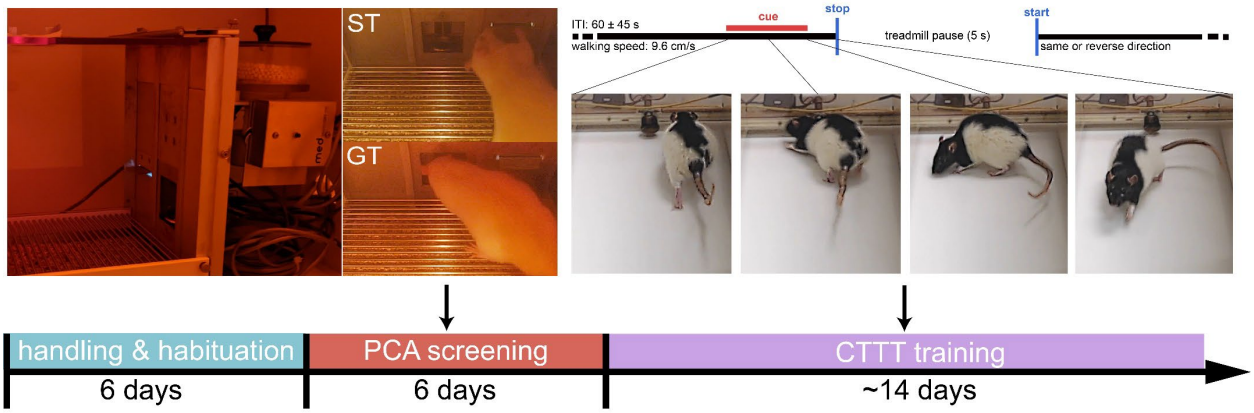
GTs and STs are sensitive to variations in the ratios of trial types. Phenotype and ratio significantly interacted (phenotype*ratio: $F_{(1,6)}$ = 10.26, p = 0.02, η_p^2 = 0.63). *Post hoc* multiple comparisons indicated that cued turn rates in GTs were significantly decreased

with 20% turn cue presentations ($F_{(2,6)} = 6.54$, $p = 0.03$, $\eta_p^2 = 0.69$, Fig. 3.5d) compared to the rates seen during 50/50 turn/stop cue sessions in these animals. *Post hoc* multiple comparisons in STs indicated that with 80% turn cue presentations STs performed significantly more cued turns ($F_{(2,6)} = 5.08$, $p = 0.05$, $\eta_p^2 = 0.63$, Fig. 3.5d) compared to 50/50 turn/stop cue sessions. Reflecting the sensitivity to trial-type manipulations in GTs and STs, the main effects of ratio and phenotype were significant (ratio: $F_{(2,6)} = 10.64$, $p = 0.01$, $\eta_p^2 = 0.78$; phenotype: $F_{(3,7)} = 4.90$, $p = 0.04$, $\eta_p^2 = 0.67$). No effects were seen with cued stops; however, there was a trending interaction between phenotype and ratio ($F_{(2,10)} = 4.01$, $p = 0.053$, Fig. 3.5e).

Decreasing the proportion of turn trials increases GLU concentrations during cued stops. No effects of varying the ratio of turn trials were seen with GLU increases during cued turns (phenotype*ratio: $F_{(2,163)} = 0.49$, $p = 0.62$). As seen in Chapter II, the GLU increases in GTs were significantly higher than in STs during cued turns (phenotype: $F_{(1,163)} = 6.34$, $p = 0.01$, $\eta_p^2 = 0.04$, Fig. 3.6d). *Post hoc* multiple comparisons indicated that cue-locked GLU increases during cued stops in sessions with 20% of stop cue presentations were significantly elevated compared to sessions with 50% and 80% stop cue presentations (ratio: $F_{(2, 67.22)} = 3.59$, $p = 0.03$, $\eta_p^2 = 0.06$, Fig. 3.6d).

Experimental timeline

Phase one screening and training



Phase two testing

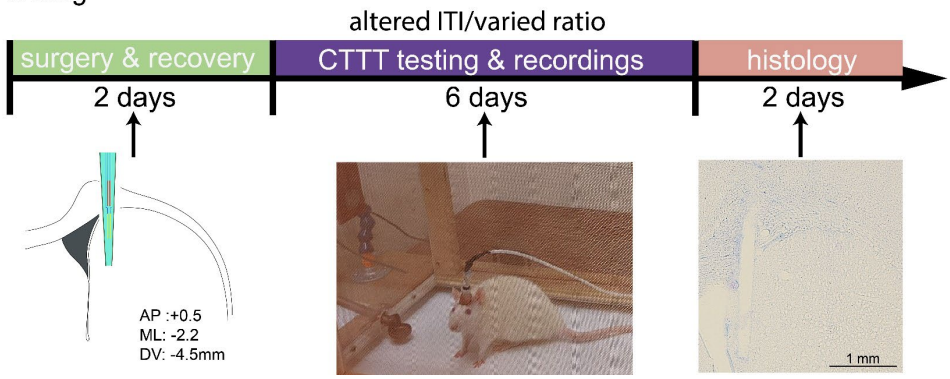


Figure 3.1: Experimental timeline for altered ITI and varied ratio of trial type manipulations
Phase 1 (screening and training): *Habituation and handling:* Rats habituated to housing chambers and were handled over 6 days. *Pavlovian conditioned approach screening*: All animals underwent 6 days of PCA screening. *Cue-triggered turning task) training*: Animals were trained on the CTTT for ~14 days.
Phase 2 (testing): *Microelectrode array implantation*: Upon reaching criterion, MEAs were chronically implanted into the right dorsomedial striatum and animals recovered over 2 days. *CTTT testing and recordings:* Amperometric recordings of test sessions were performed over 6 days. *Histology:* Tissue processing to confirm MEA placement was carried out over 2 days.

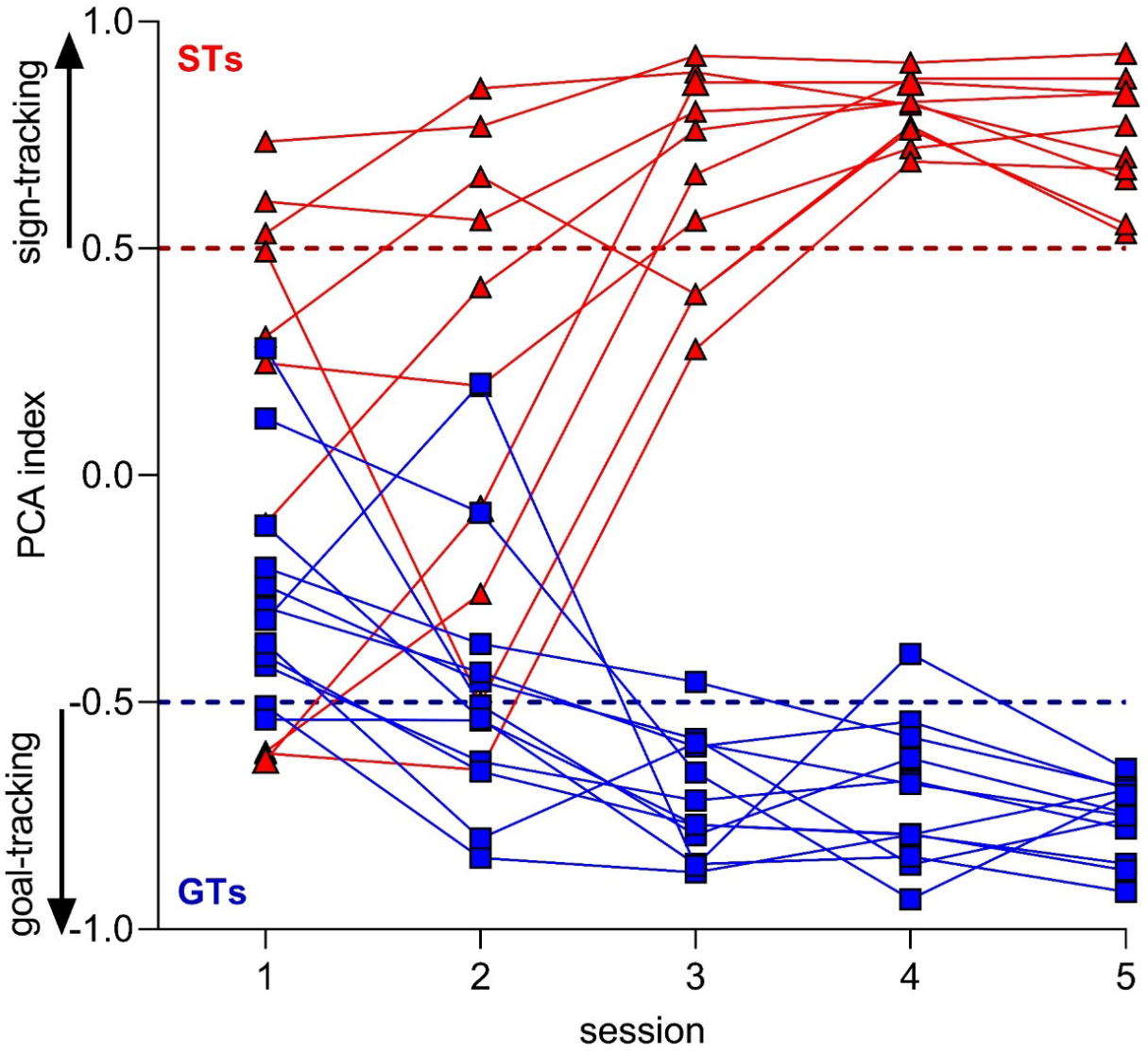


Figure 3.2: Distribution of GTs and STs used for altered ITI and varied ratio experiments. The phenotypes of goal-tracking rats (blue squares) and sign-tracking rats (red triangles) were assessed across 5 Pavlovian Conditioned Approach sessions (x-axis). Scores averaged from sessions 4 and 5 were used to assign phenotype with predetermined cutoffs of 0.5 (red dotted line) for STs and -0.5 (blue dotted line) for GTs on the y-axis.

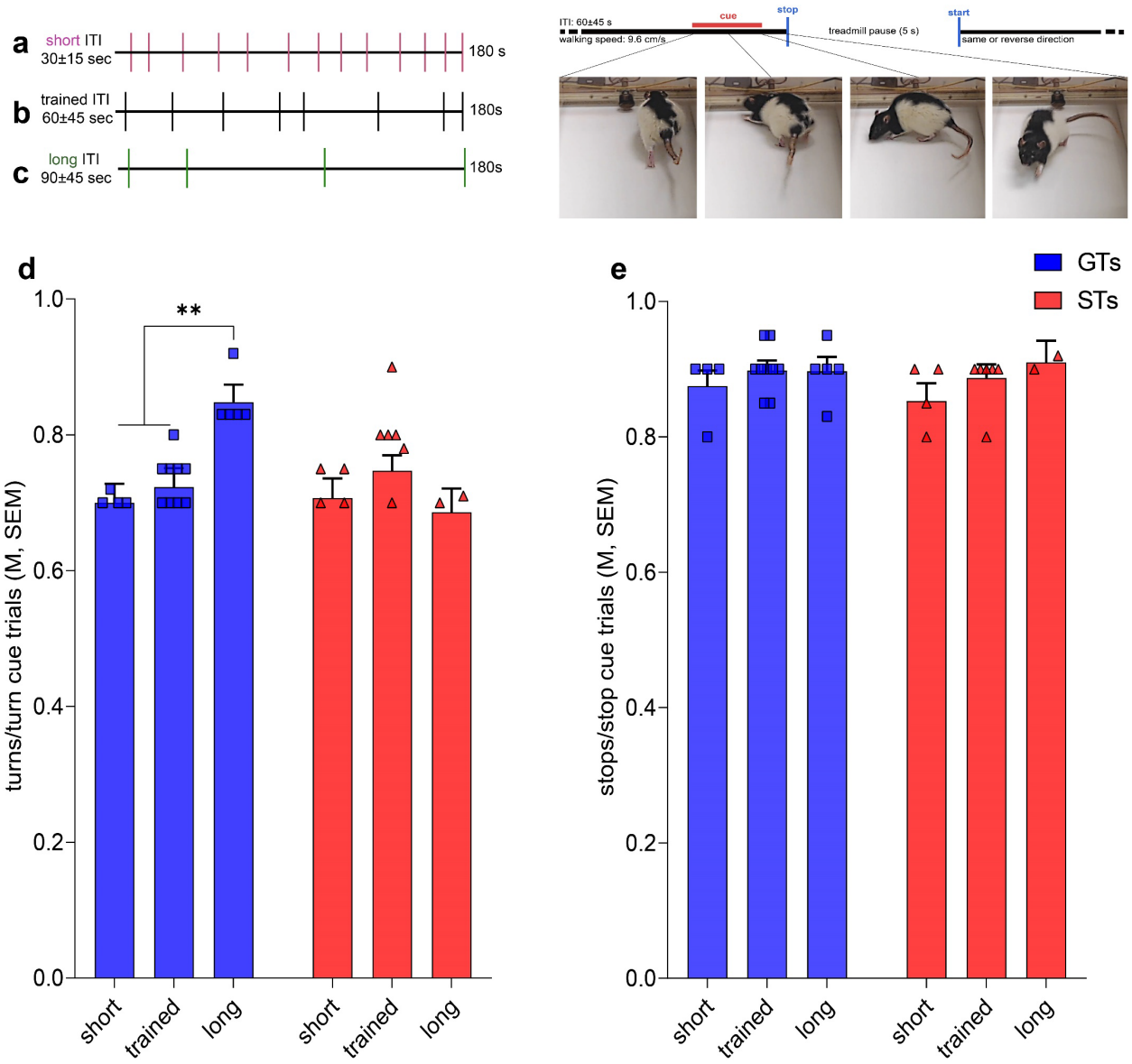


Figure 3.3: The effects of altering the ITI on cued turn and stop rates

a-c: Altered inter-trial interval (ITI) durations. CTTT consists of 18 stop and turn trials with an ITI of (a) 60±45 s. For test conditions, the ITI was shortened (short) to 30±15 s (b) or lengthened to 90±45 s (c). d: The effects of shortening or lengthening the ITI on the number of turns per turn cue trials (represented as EMM±SEM with individual values superimposed) in GTs (blue) and STs (red). Extending the ITI, significantly increased the number of cued turns in GTs. Cue-triggered turning in STs was unchanged by altering the ITI. e: The effects of shortening or lengthening the ITI on the number of stops per stop cue trials in GTs and STs. The rates of cued stops in GTs and STs were unchanged by altering the ITI. Post-hoc comparisons between ITIs in GTs: $p<0.01$**.

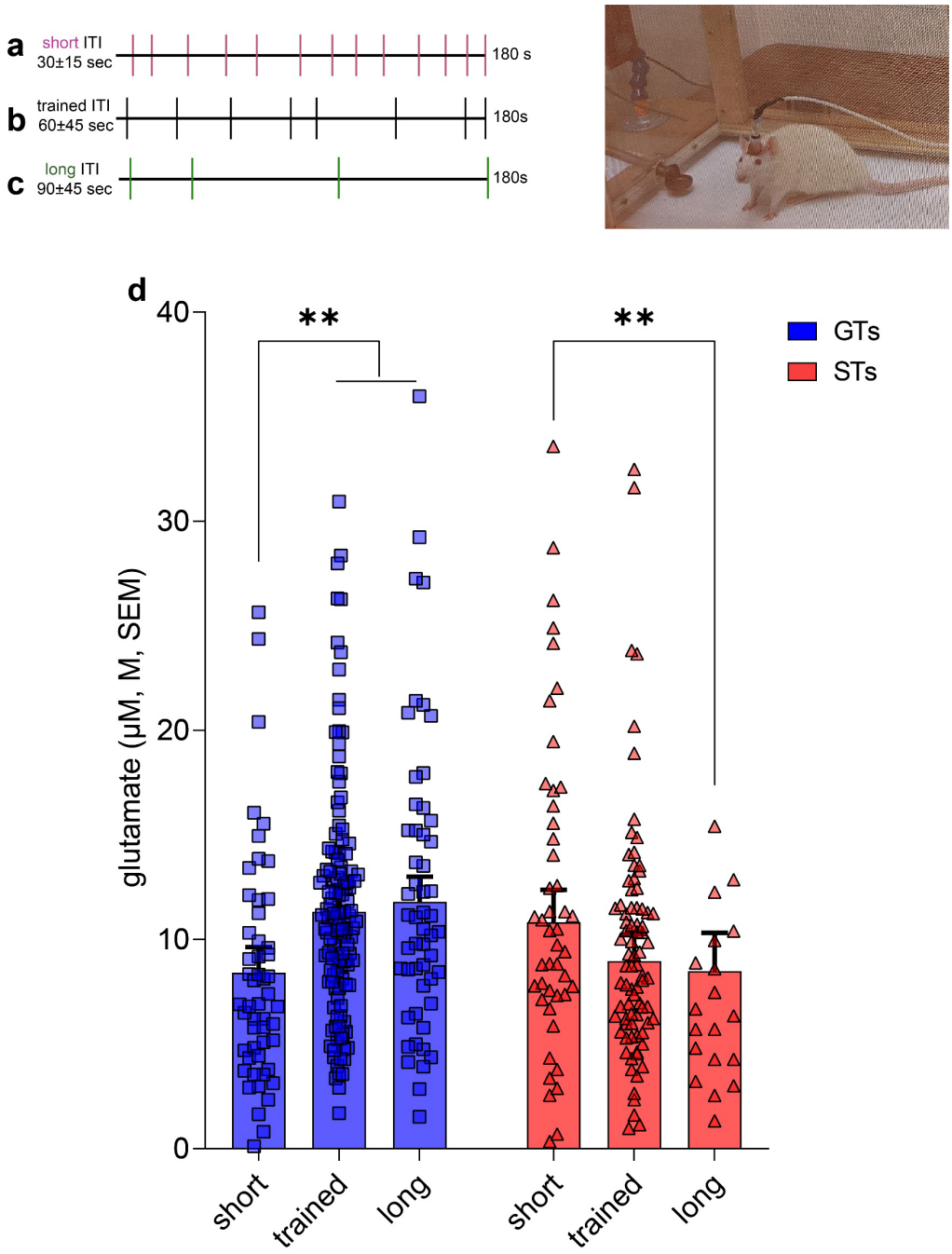


Figure 3.4: Opposing impact of ITI length on GLU concentrations GTs and STs
a-c: Altered inter-trial interval (ITI) durations. CTTT consists of 18 stop and turn trials with an ITI of (a) 60±45 s. For test conditions, the ITI was shortened (short) to 30±15 s (b) or lengthened to 90±45 s (c). d: Individual peak glutamate (GLU) concentrations (represented as EMM±SEM with individual values superimposed) during cued turns in GTs (blue) and STs (red) during sessions with the trained, short, or long ITI conditions. Amplitudes of GLU concentrations in GTs (blue bar) during the short ITI condition were significantly smaller compared to the trained and long ITI conditions. GLU amplitudes in STs (red bar) from short ITI sessions were significantly larger than amplitudes from the long ITI sessions. Glutamate concentration in µM displayed on the y-axis. Post-hoc comparisons between ITIs within each phenotype: $p<0.01$**.

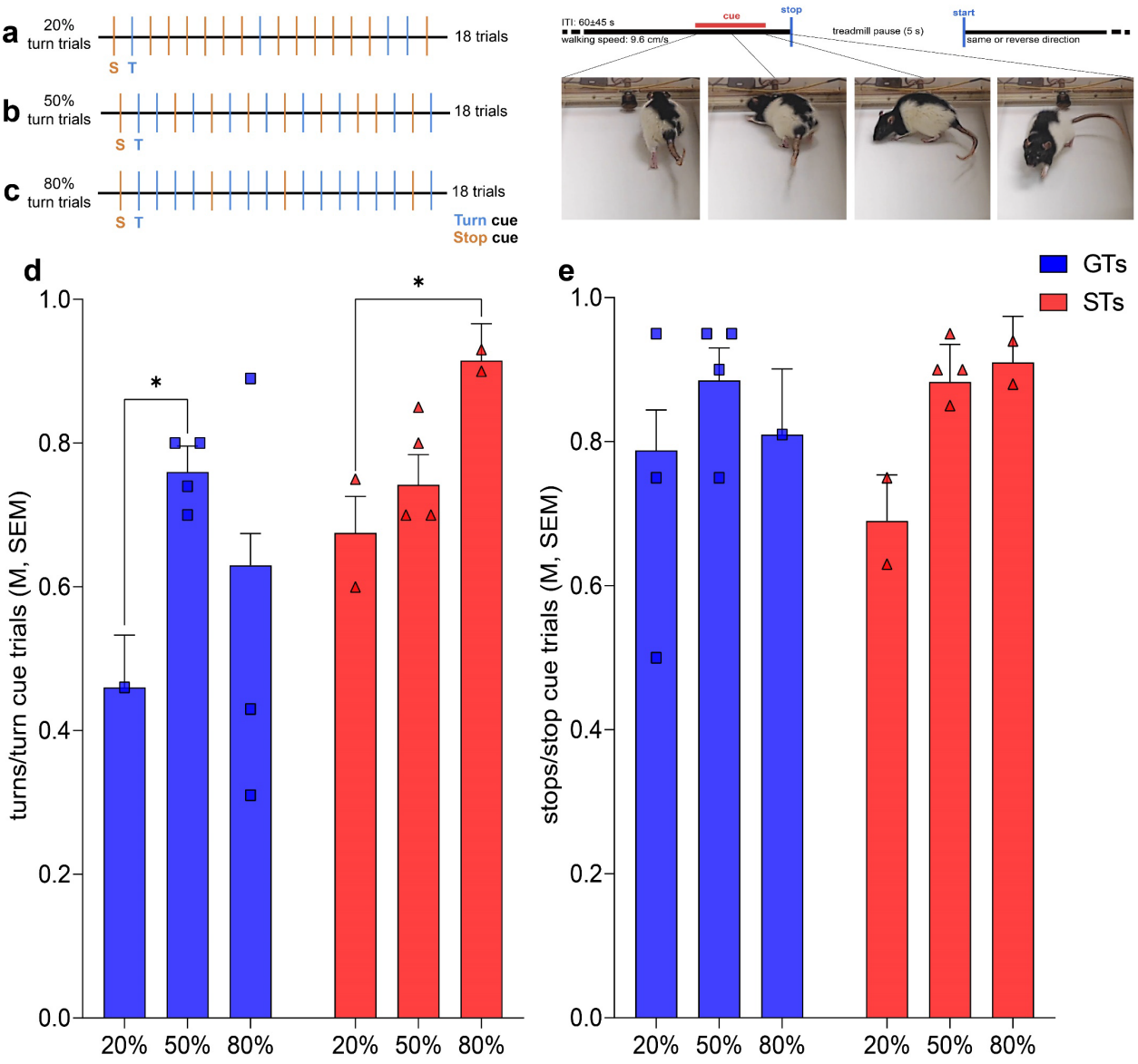


Figure 3.5: Differential impact of turn trial ratios on cue-triggered turning in GTs and STs
a-c: Varied trial type ratios. CTTT consists of 50% (a) turn and stops trials (9 of each). For test conditions, the ratio of turn trials was decreased to 20% (b) or increased to 80% (c) approximately 3 and 14 turn trials, respectively. d: The effects of decreasing or increasing turn trials (represented as EMM±SEM with individual values superimposed) in GTs (blue) and STs (red). Decreasing the proportion of turn cue presentations decreased the number of cued turns in GTs. The rates of cued turns in STs with 20% of turn trials was significantly lower than in STs with 80% of turn trials. e: The effects of decreasing or increasing of stop trials in GTs and STs. No effects of varying the proportion of stop trials on the rates of cued were found in GTs or STs. Post-hoc comparisons between ratios within each phenotype: $p<0.05$*.

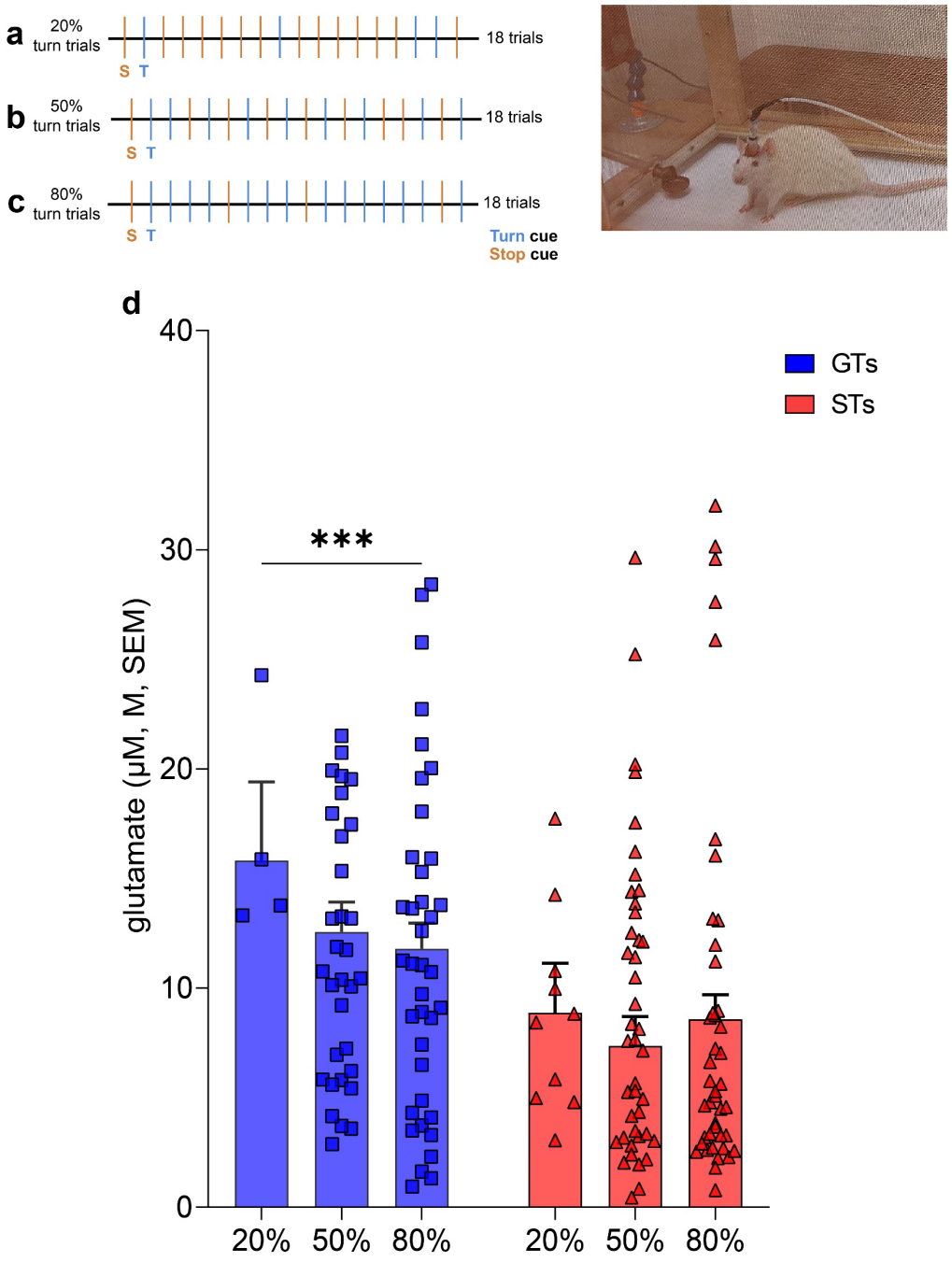


Figure 3.6: No effect of turn trial ratio on GLU increases during cued turns in GTs and STs a-c: Varied trial type ratios. CTTT consists of 50% (a) turn and stops trials (9 of each). For test conditions, the ratio of turn trials was decreased to 20% (b) or increased to 80% (c) approximately 3 and 14 turn trials, respectively. d: Increases in glutamate (GLU) concentrations (represented as EMM±SEM with individual values superimposed) during cued turns in GTs (blue) and STs (red) with error bars indicating the mean and standard error of the mean during sessions with the proportion of turn trials decreased (20%) or increased (80%). GLU increases in GTs were significantly larger than STs, but unaffected by the ratio of turn trials. ***above GTs indicates a main effect (p<0.01) of phenotype. Glutamate concentration in µM displayed on the y-axis.

Chapter IV: The Role of Corticostriatal Inputs in Cue-Triggered Turning in GTs

Abstract

Using a dual-vector approach to silence corticostriatal projections, we verified the cortical origin of the GLU activity recorded in these experiments. Moreover, silencing this pathway increased the number of misses in GTs, not STs. This evidence supports the hypothesis that cortico-striatal transfer of movement cues is essential for cue-guided movement. However, in rats exhibiting poor attentional control as a trait (STs), such transfer may be functionally replaced by bottom-up modulation of GLU release. Such “replacement” may be adequate for performing well-practiced, cued movements but is predicted to disrupt performance in unfamiliar and dynamic environments. Upon corticostriatal silencing, peak GLU amplitudes and cue-triggered turning were attenuated in GTs but not STs, further supporting that STs do not engage this top-down circuitry.

Introduction

Everyday activities often become second nature, seamlessly integrated into our ongoing cognitive processes. However, occasional errors disrupt the harmony between planned and executed movements. These disruptions trigger an attentional shift, directing our focus toward the essential cues for corrective actions. In dynamic situations requiring such shifts, cortical cholinergic transients may contribute to the attentional-motor integration within the striatum through glutamatergic interactions, updating the circuitry necessary for executing responses guided by past response-outcome associations. In *Chapter II*, we conducted real-time measurements of glutamate (GLU) levels in the dorsomedial striatum (DMS) of animals with varying cortical cholinergic modulation capacities (GTs and STs) performing a cue-triggered turning task (CTTT). We observed increases in cue-locked glutamate (GLU) associated with cue-triggered turning in GTs, supporting our hypothesis that movement-related cues elicit dorsomedial striatal GLU. Interestingly, GTs showed lower cue-locked glutamate concentrations during stop trials, further indicating that dorsomedial GLU is secondary to transient cortical cholinergic

activity. This finding aligns with the requirement of external cue detection (turn cue) for cued turning in the CTTT, leading to the interruption and reprogramming of motor sequences. To assess the necessity of cortical inputs and to confirm the source of glutamate in the DMS during cue-triggered turning in GTs, we employed a dual-vector approach to silence the corticostriatal pathway in both GTs and STs while measuring real-time dorsomedial striatal GLU levels. We predicted decreases in cued turns and GLU levels in GTs, but not STs, given the absence of cortical cholinergically mediated cue detection in these animals.

STs approach and work for the presentation of reward-predictive cues, while GTs apply the utility of these cues to direct goal-oriented behaviors. (Flagel & Robinson, 2017; Meyer et al., 2012; Robinson & Flagel, 2009). Approaches to reward-predictive cues may implicate attentional and motivational processing dependent on the relative salience of these cues. The distinct behaviors and biases to predictive versus discriminative cues instigating motivated-responding highlight opposing cognitive-motivational styles in STs and GTs (Pitchers et al., 2017a; Sarter & Phillips, 2018). Behaviors in STs instigated by reward-associated cues are primarily driven by incentive motivational processes, which may implicate a relatively weak degree of top-down attentional control (Berridge, 2000; Berridge & Robinson, 2003; Iglesias et al., 2020). In line with this perspective, STs display cognitive inflexibility when challenged and heightened distractor vulnerability, thought to be mediated by limited capacities to maintain acetylcholine (ACh) release (Ahrens et al., 2016; Flagel et al., 2009; Koshy Cherian et al., 2017; Kucinski et al., 2018; Paolone et al., 2013; Pitchers et al., 2017c). In contrast, in tasks employing reward-predictive cues, GTs exhibit fewer approaches but still orient to the cue (Meyer et al., 2012; Pitchers et al., 2017b). Conversely, the processing of contextual discriminative cues in GTs is cholinergically dependent, reflecting a propensity to engage processes underlying cue detection. Here, “detection” refers to the heightened signal processing that triggers cue-directed responses and informs the system about the accuracy or outcome of performing such responses (Posner, 1980). Transient phasic cholinergic signals in the prelimbic cortex referred to as "transients," initiate attentional shifts from cue monitoring to cue detection (Gritton et al., 2016; Howe et al., 2017). Therefore, GTs may engage attentional cue processing that integrates contextual and complex information to drive goal-oriented

action (Morrison et al., 2015; Pitchers et al., 2017a; Pitchers et al., 2017b; Sarter & Phillips, 2018).

These findings align with research investigating the role of corticostriatal in action-outcome associations. Human fMRI studies, such as those by Chatham et al. (2014), indicate that frontostriatal circuits selectively gate relevant working memory information to guide future behavioral responses, indicating a role in updating learned response-outcome associations. This concept is corroborated by simultaneous local field potential (LFP) recordings from the frontal cortical and dorsomedial striatal regions, revealing a progressive increase in activity during cue presentation to the execution of responses, followed by a subsequent decline in activity upon reward delivery (Kim et al., 2015; Vandaele et al., 2021a). These recordings suggest a potential role of this activity in mediating outcome evaluations through temporal links between actions and consequences. Consistent with these findings, inactivating the corticostriatal pathway impairs action-outcome learning and expression (Hart et al., 2018; Kimchi et al., 2009; Morris et al., 2015).

Based on these findings, we hypothesize that GTs rely on engaging fronto-cortical cholinergic processes to guide cue-triggered goal-directed action. Additionally, we hypothesize that corticostriatal transfer of movement cues, signified by dorsomedial glutamate concentrations, is essential for this cue-guided movement in GTs. Employing a dual-vector approach to silence corticostriatal projections, we conducted amperometric recordings in the dorsomedial striatum of GTs and STs performing a cue-triggered turning task. Interestingly, in STs, rats typified by poor top-down attentional control, no impacts on CTTT performance or cue-locked GLU levels were observed. In STs, glutamate release may result from modulatory sources and serve to guide well-practiced, cued movements. In GTs, peak GLU amplitudes and cue-triggered turning were attenuated upon corticostriatal silencing. These results implicate corticostriatal inputs as the source of dorsomedial glutamate increases during cue-triggered turning in GTs, whereupon removing this activity disrupts performance.

Methods

Subjects. 171 Sprague Dawley rats (obtained Inotiv, West Lafayette, IN or Taconic, Rensselaer, NY; n = 93 females and n = 78 males; 250-500g) were individually housed

on a 12-hour light/dark cycle (lights on at 7:00 AM) at ~21ºC with ad libitum access to food (Laboratory Rodent Diet 5001, LabDiet) and water. Three cohorts of rats were used in the experiments detailed below (n = 43, 19 females). The first cohort was obtained from Taconic exclusively; cohorts 2 and 3 were mixed between Taconic and Inotiv. The remaining animals were used for other experiments. All experimental procedures were approved by the University Committee on the Use and Care of Animals (UCUCA) at the University of Michigan and carried out in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care. Animals acclimated to housing quarters for two days prior to handling and manipulation.

Experimental timeline. Figure 4.1 illustrates the timeline of experiments described in this study. Initially, rats underwent 6 days of habituation to housing facilities and experimenters through regular handling. Following habituation, rats were screened for goal-tracking and sign-tracking phenotypes for another 6 days. Subsequently, they underwent dual-vector infusion surgeries. After a 7-day recovery period, cue-triggered turning task (CTTT) training was conducted for approximately 14 days. Once the rats met the training criterion, they received injections of either vehicle or clozapine n-oxide (CNO), administered 50 minutes before CTTT testing. For a subset of rats, microelectrode arrays were implanted into their right dorsomedial striatum. After a 2-day recovery period, these rats received either vehicle or CNO injections 50 minutes before performing the CTTT, while simultaneous amperometric recordings were taken. Subsequently, brains were harvested, and coronal sections were taken to confirm the proper placement of the microelectrode arrays. Detailed procedures used in *Chapter II* can be found in that chapter, while the procedures exclusive to these experiments are described below.

Dual-vector infusion of Cre-dependent DREADDs and retrograde-Cre vectors. To test the hypothesis that environmental cues evoke corticostriatal glutamatergic signaling to guide complex movement, we utilized a pathway-specific dual-vector chemogenetic strategy to selectively silence the activity of cortical inputs to the projection field of the dorsomedial striatum (diagrammed in Experimental Timeline). The viral vectors containing the Cre-dependent plasmid pAAV-hSyn-DIO-hM4D(Gi)-mCherry (AddGene #44362-AAV8; titer of 2.1x1013 GC/mL), Cre-dependent control plasmid pAAV-hSyn-DIO-mCherry (AddGene #50459-AAV8; titer of 2.2x1013 GC/mL), or the retrograde Cre-expressing

plasmid pENN-rAAV-hSyn-HI-eGFP-Cre-WPRE-SV40 #105540-AAVrg; titer of 1.9x1013 GC/mL) were obtained from Addgene. The surgical techniques and conditions described in *Chapter II* were implemented here. Craniotomies and durotomies were performed above four sites of the dorsomedial striatum using a bent 27-gauge needle for dura removal to minimize damage for later recordings. One µL of pENN-rAAV-hSyn-HI-eGFP-Cre-WPRE-SV40 vector was infused (bolus) into the dorsomedial striatum at two sites per hemisphere (AP: +0.2/1.2; ML: ±2.5/2.2; and from the skull: DV: -4.5mm) to retrogradely infect projections neurons while one µL of AAV-hSyn-DIO-hM4D(Gi)-mCherry or AAV-hSyn-DIO-mCherry (control) vector was infused (bolus) into the frontal cortex (AP: +3.2; ML: ±0.7, and from the skull: DV: -3.5mm) to silence corticostriatal projections selectively. The injector was left in place for 8 minutes to minimize diffusion into the injector tract. Rodents recovered for 1 week before treadmill and CTTT training.

CNO administration. CNO was obtained from Tocris Bioscience (Bristol, United Kingdom) and dissolved 10 mg/ml in 6% DMSO in 0.9% NaCl solution. Intraperitoneal injections (i.p.) of vehicle or CNO were administered at a dose of 5.0 mg/kg 50 min before CTTT testing or at the start of baseline for amperometric recordings. Vehicle and CNO injections were alternated each day. Previous work reported the potential off-target effects due to CNO conversion to clozapine (Gomez et al., 2017). However, others and we previously found no impact of CNO at this dose in control construct-expressing rats performing the CTTT, other movement tasks, and attention tasks (Avila et al., 2020; Kucinski et al., 2022; Lawson et al., 2023). Additionally, using the proposed conversion rate of CNO to clozapine, a 50-100-fold higher dose of clozapine would be required to produce direct neurochemical and behavioral effects (Mahler & Aston-Jones, 2018; Mahler et al., 2019; Martinez & Sarter, 2008; Martinez et al., 2019).

Statistical analyses. Linear mixed-effects models were used for analyses (rationale detailed in Chapter II). For CTTT performance measures and electrochemical data, repeated sessions with injection (CNO or vehicle) were used as the repeated variable, and phenotype, modality, and sex were used as the between-subjects variables with the subject identifier as a random intercept.

mCherry amplification and GFP/mCherry-labeling. As stated, we utilized a dual-vector approach to target corticostriatal projections selectively (Fig. 4.1). We amplified the mCherry fluorescent signal from the inhibitory hM4Di vector to evaluate the transfection efficacy and distribution of mCherry and GFP expression in the frontal cortex. The eGFP fluorescent label of the retrograde Cre virus signal was strong and did not necessitate signal enhancement. Sections underwent six washes for 5 min each in 0.1 m PBS, pH 7.3, and then immersed in 0.1% Triton X-100 diluted in PBS for 15 min. After three 5-min PBS rinses, sections incubated for 60 min at room temperature (RT) in the blocking solution, 1% normal donkey serum (NDS) and 1% Triton X-100 made in PBS. The sections incubated overnight in the primary antibodies (rabbit anti-mCherry, ab167453, Abcam; 1:500). All primary antibodies (incubations were diluted in blocking solution) to prevent non-specific binding. The next day following three 5-min PBS rinses, sections incubated for 90 min at RT in the secondary antibody (donkey anti-rabbit conjugated to Alexa 594, PIA32754, Invitrogen, 1:500). Following three 5 min-rinses with PBS and sections were mounted, air dried, and cover-slipped with Vectashield Antifade Mounting Medium (H-1000; Vector Laboratories).

A Zeiss LM 700 confocal microscope, equipped for sequential multi-track acquisition with 488- and 561nm excitation lines, along with specific filter sets for Alexa 488 (Zeiss filter set 38 HE) and Alexa 594 (Zeiss filter set 54 HE), was employed for visualizing and capturing images of fluorescent neurons. Images were taken at magnifications of 10× (to confirm placement in the dorsomedial striatum), 20× (for verification and documentation of double-labeled cells), and 40× at five anterior-posterior levels (frontal cortex: 3.0 mm and 3.24 mm, striatum: 1.2 mm, 0.5 mm, and 0.2 mm). Cre-positive cells expressing GFP in the dorsomedial striatum were used to generate counts based on the placement and size of the transfection space (Fig. 4.7a).

Transfected areas centered in and restricted to the dorsomedial striatum were assigned the highest score (5), whereas more lateral and areas that spread further were assigned ≤4. Sections were also used to confirm the placement of the MEAs at AP 0.5 mm. Single-labeled and double-labeled neurons were counted in four subregions of the frontal cortex [PL, IF, CG, M1/2]. Counting frames were superimposed on each subsection (PL, IF, CG, M1/2), and counts, using the ImageJ multipoint tool, were restricted to these frames.

Counts were developed based on two sections per rat, yielding four counts per rat and subregion. Averaged counts obtained from each brain and subregion were used for further analyses.

For imaging, tile scanning/stitching techniques were implemented at both 10× (3 × 2 tiles, covering an area of 3455.94 by 2370.64 μm) and 20× (4 × 3 tiles, covering an area of 1407.88 by 1135.31 μm) magnifications. The split view feature within Zen Black software allowed for examining individual channels with multi-track images. For counts, double-labeled neurons representing DREADD-expressing neurons of the PL projecting to the dorsomedial striatum were designated as cells expressing green (Alexa 488) and red (Alexa 594) fluorescence. We ran simple linear regressions of cell counts against the difference in cue-triggered turns and stops with CNO or saline injected to determine if DREADD-induced behavioral changes in hM4Di rats correlated with transfection efficacy scores and the percent of double-labeled cells. Sections from all phenotypes, including STs, were processed, and cell counts were compared against CNO-associated performance changes to test for the possibility that CNO's relative lack of effect in STs was due to DREADD expression inefficacy.

Results

Cue-triggered turning in GTs but not in STs is disrupted by silencing the corticostriatal pathway. Separate linear mixed-effects models (LMMM) were used to test the effects of CNO or vehicle in rats expressing the inhibitory hM4Di DREADD (n=21, 13 females) or only mCherry (n=9, 4 females). CNO administration in rats expressing the inhibitory DREADD significantly reduced the number of cued turns during trials with turn cue presentation in GTs but not STs (phenotype x injection: $F_{(1,17)} = 22.06$, $p < 0.001$, $\eta_p^2 = 0.24$; Fig 4.3b). The main effects of phenotype and injections (vehicle/CNO) were also significant ($F_{(1,17)} > 7.12$ and $p < 0.02$ for both). Cued stop rates did not differ between phenotype and injection in rats expressing hM4Di (phenotype x injection: $F_{(1,17)} = 3.52$, $p = 0.08$, Fig. 4.3c). Importantly, in rats expressing the mCherry control construct, cued turn and stop rates did not significantly differ in GTs and STs, with vehicle or CNO onboard ($F < 1.75$, $p > 0.23$ for all; Fig. 4.4).

Effects of inhibitory DREADD activation on GLU in GTs and STs. A subset of GTs and STs (n=15, 8 GTs, 6 females) had microelectrode arrays (MEAs) chronically implanted into their dorsomedial striatum to be recorded while performing the CTTT. For these recordings, the MEAs had slopes of 12.04 ± 0.22 pA/µm, a selectivity over ascorbic acid (glutamate: AA) of 113.12 ± 3.53, limit of detection of 0.34 ± 0.02 µm glutamate, and highly linear responses of 0.98 ± 0.001 (see Amperometric recordings of glutamate currents for details of MEAs calibration). Linear mixed-effects models (LMMM) were used to test the effects of CNO or vehicle on glutamate concentrations in these rats with inhibitory hM4Di DREADD (n=13, 5 females) or only mCherry (n=2, 1 female).

Post hoc multiple comparisons indicated that CNO administration significantly attenuated GLU concentrations in GTs but not STs ($F_{(1,73)} = 26.65$, $p < 0.001$, $\eta_p^2 = 0.27$; Fig. 4.5b). Reflecting the profound effect of CNO administration on glutamate increases in GTs expressing the inhibitory DREADD, the main impact of injection (injection: $F_{(1,197.54)} = 40.79$, $p < 0.001$, vehicle: 10.34 ± 0.52, CNO 5.87 ± 0.60) also reached significance. These increases peaked in seconds slower in STs at baseline (vehicle); however, CNO administration increased the time to peak in GTs such that the GTs and STs were equivalent (phenotype*injection: $F_{(1,73)} = 4.44$, $p = 0.04$, $\eta_p^2 = 0.06$; Fig. 4.5c). The number of peaks in STs was higher than GTs with both injections; however, the GLU increases in GTs peaked more often with CNO administration (phenotype*injection: $F_{(1,73)} = 3.83$, $p = 0.04$, $\eta_p^2 = 0.05$; Fig. 4.5d). Importantly, CNO administration did not impact GLU concentrations in GTs and STs expressing only mCherry ($F < 0.96$, $p > 0.33$, for all). GLU increases in GTs during cued stops were significantly lower than in STs but unchanged by CNO administration (phenotype: $F_{(1,9.77)} = 5.56$, $p = 0.04$; Fig. 4.6b). Transient GLU increases in STs peaked less than in GTs during cued stops (phenotype: $F_{(1,8.59)} = 15.54$, $p < 0.001$; GTs: 1.58 ± 0.05, STs: 1.29 ± 0.05; Fig. 4.6d).

In hM4Di-expressing GTs, CNO administration significantly attenuated increases in glutamate concentrations in GTs but not STs. GLU concentrations associated with cued-stop trials were lower in GTs compared to STs, but not impacted by CNO administration. GLU concentrations in STs during cued stops increased faster and peaked less compared to GTs, replicating findings in *Chapter II.* In summary, the results indicate distinct effects of CNO administration on GLU in GTs and STs, suggesting the importance of

corticostriatal inputs in cue-guided movement exclusively in GTs. The differences in GLU between GTs and STs highlight the heterogeneity in their neural circuitry and cognitive-motivational styles during the CTTT.

mCherry and GFP expression in the frontal cortex and caudate nucleus. As detailed in the methods, a retrograde Cre vector tagged with GFP was infused into the dorsomedial striatum, while the inhibitory DREADD tagged with mCherry was infused into the frontal cortex to allow CNO-induced activation of the inhibitory DREADD expressed in frontal neurons projecting to the dorsomedial striatum. The total number of positive cells in the frontal cortex and the striatum were classified into five classes with scores for the prelimbic cortex (PL) and the dorsomedial striatum (DMS); Fig. 4.7a. The total number and proportion of GFP-positive cells were counted in four regions of the striatum (DM, DL, VM, VL; Fig. 4.7b). The total number and proportion of mCherry-positive cells were counted in three subregions of the frontal cortex projecting to the striatum (PL, IF, CG; Fig. 4.7c). The transfection efficacy in the PL but not the DMS were significantly correlated with CNO-induced decreases in cued turns in GTs (PL: $R^2 = 0.49$, $F = 8.37$, $p = 0.02$; Fig. 4.7d-e).

Microphotographs of the frontal cortex shown in Figure 4.8*a-d* show GFP-positive, mCherry-positive, and neurons co-expressing the retrograde virus and inhibitory DREADD. STs did not show cue-triggered turning deficits with CNO administration, and therefore, were not included in correlational testing. Sections from GTs and STs were compared to ensure that the lack of CNO effects in STs was not due to poor transfection efficacy. Transfection efficacy did not differ between phenotypes (no main effect of phenotype or region x phenotype, $F < 0.82$, $p > 0.50$ for both). Expression was restricted primarily to the PL for all animals (region: $F_{(2,50)} = 161.54$, $p < 0.001$, $\eta_p^2 = 0.87$; PL: 0.68 ± 0.02, IF: 0.10 ± 0.02, and CG: 0.22 ± 0.02). The ratio of double-labeled cells restricted to the PL of GTs was significantly correlated with the difference in cued turns/turn trials ($R^2 = 0.77$, $F = 29.35$, $p < 0.001$, Fig. 4.8f-g*).* The ratio of double-labeled neurons in the IF and CG were not correlated with CNO-induced decreases in cued turns. Altogether, DREADD transfection efficacy in the PL predicted behavioral deficits exclusively in GTs. Sections from rats chronically implanted with MEAs were taken to confirm placement in the dorsomedial striatum.

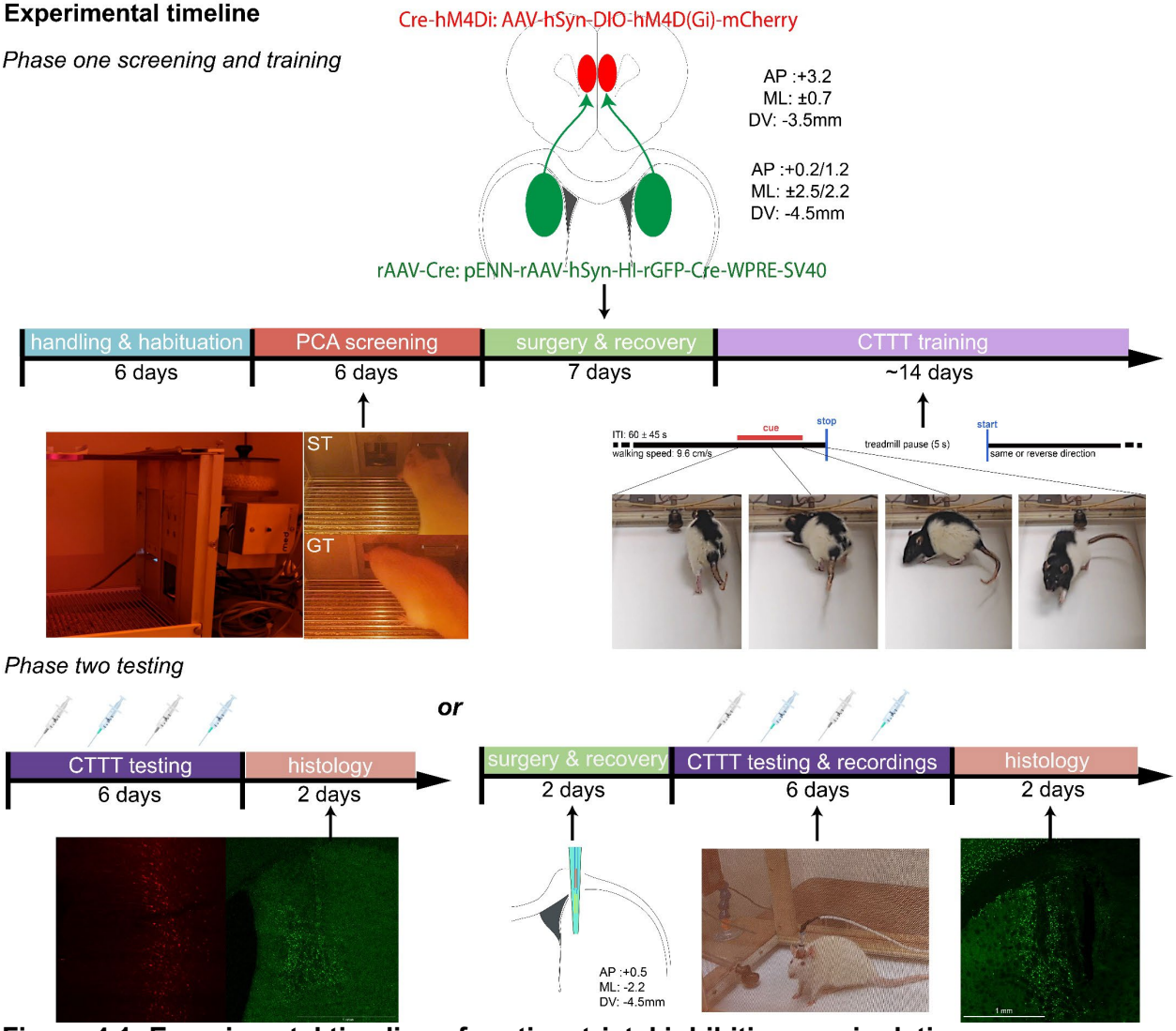


Figure 4.1: Experimental timeline of corticostriatal inhibition manipulation

Phase 1 (screening and training):

Habituation and handling: Rats habituated to housing chambers and were handled over 6 days. *Pavlovian conditioned approach screening*: All animals underwent 6 days of PCA screening. *Dual-vector infusions*: Animals received inhibitory DREADD infusion into the frontal cortex and retrograde-Cre viral infusion into the dorsomedial striatum (DMS). *Cue-triggered turning task training*: Following 7 days of recovery, animals trained on the CTTT for ~14 days.

Phase 2 (testing):

CTTT testing: Test sessions with alternating days of vehicle or CNO injections occurred over 6 days. *Histology:* Tissue processing to confirm MEA and viral placements was carried out over 2 days. *Microelectrode array implantation*: Upon reaching criterion, MEAs were chronically implanted into the right DMS and animals recovered over 2 days. *CTTT testing and recordings:* Amperometric recordings of test sessions were performed over 6 days. *Histology:* Tissue processing to confirm MEA and viral placements was carried out over 2 days.

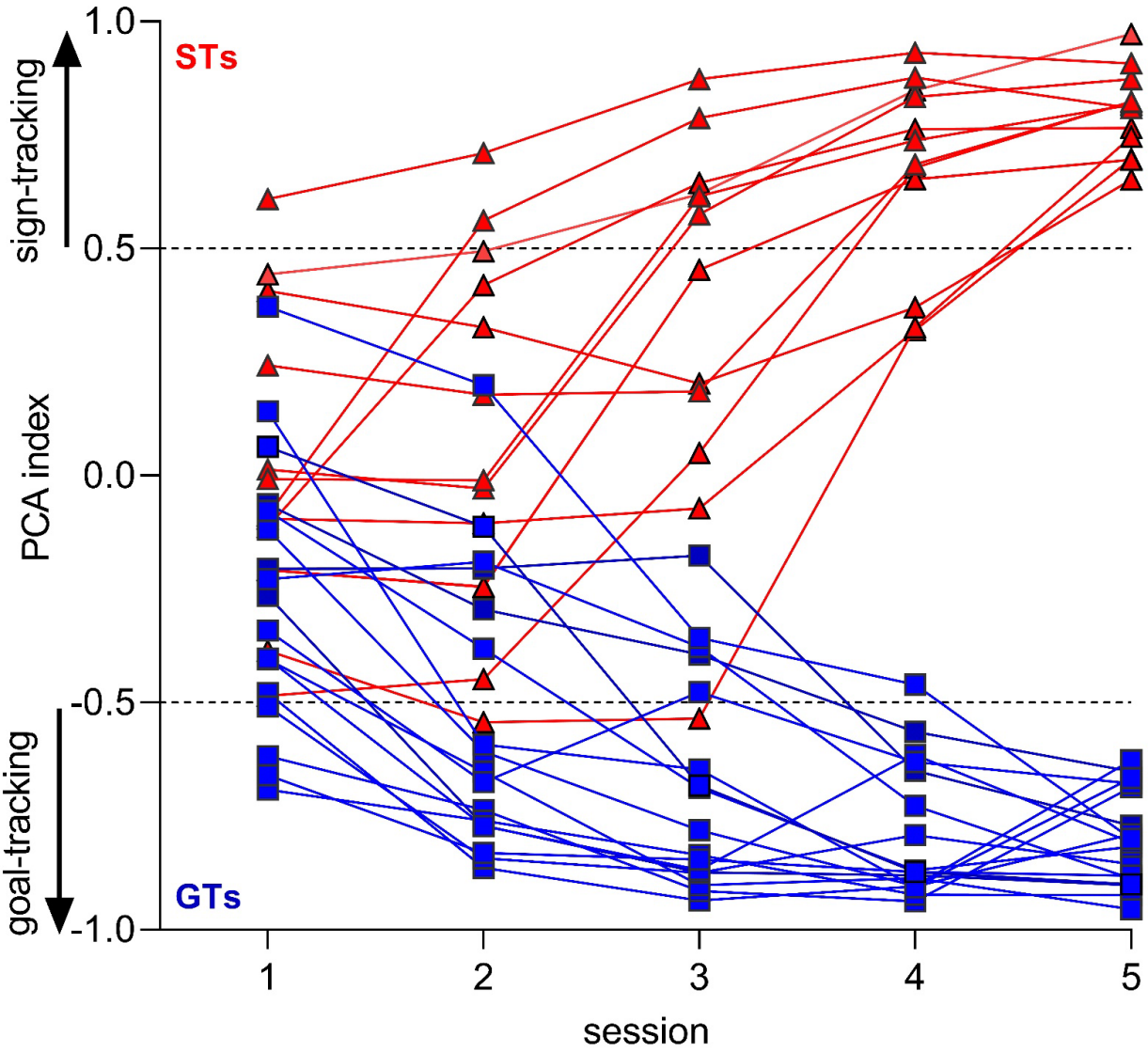


Figure 4.2: Distribution of GTs and STs used for corticostriatal inhibition experiment
The phenotypes of goal-tracking rats (blue squares) and sign-tracking rats (red triangles) were assessed across 5 Pavlovian Conditioned Approach sessions (x-axis). Scores averaged from sessions 4 and 5 were used to assign phenotype with predetermined cutoffs of 0.5 (red dotted line) for STs and -0.5 (blue dotted line) for GTs on the y-axis.

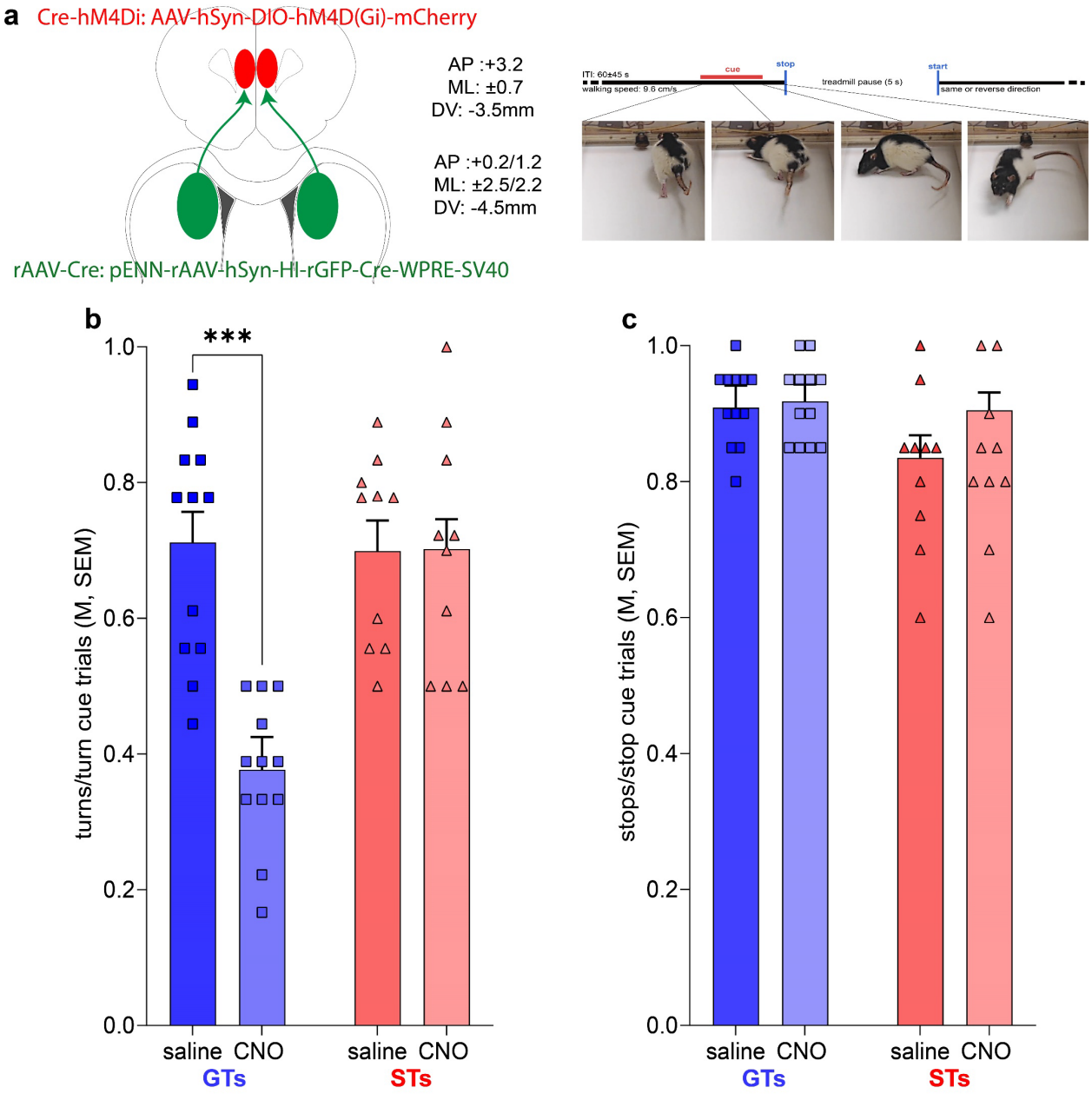


Figure 4.3: CNO administration decreases cue-triggered turning in hM4Di GTs

a: Dual-vector scheme; an inhibitory Cre-dependent virus was bilaterally infused into the prelimbic cortex, while a retrograde Cre vector was bilaterally infused into dorsomedial striatum. b: The proportion of cued turns (represented as EMM±SEM with individual values superimposed) in trials presenting the turn cue was significantly lower in CNO-treated, hM4Di-expressing GTs (purple) when compared to the effect of vehicle (blue) in these same animals and in hM4Di-expressing STs with vehicle (red) or CNO (pink) administered. c: The proportion of cued stops in trials presenting the stop cue was not significantly different in hM4Di-expressing GTs or STs with vehicle or CNO administration. Post-hoc comparisons between injections within GTs: $p<0.001$***.

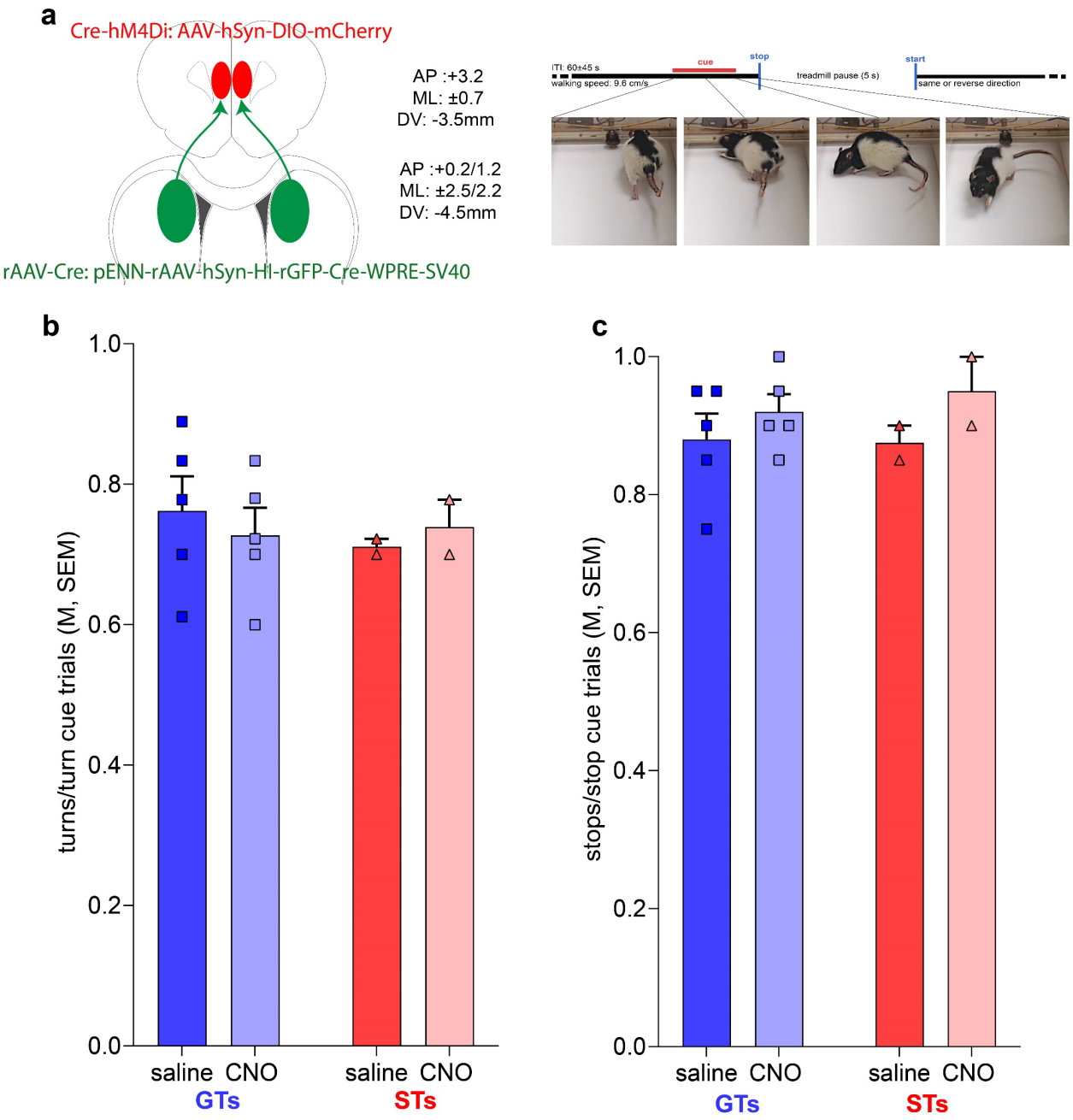


Figure 4.4: No effects of CNO administration on turns or stops in control animals
a: Dual-vector scheme; an inhibitory Cre-dependent virus was bilaterally infused into the prelimbic cortex, while a retrograde Cre vector was bilaterally infused into dorsomedial striatum. b: Individual turn scores of GTs during sessions with vehicle (blue) or CNO (purple) and STs (red) or CNO (pink) injected peripherally. c: Individual stop scores of GTs and STs during sessions with vehicle (saline) or CNO injected peripherally. The proportions of cued turns and stops in turn cue or stop cue trials were not significantly different in mCherry/GFP-expressing controls with vehicle or CNO administration. All data represented as M ± SEM.

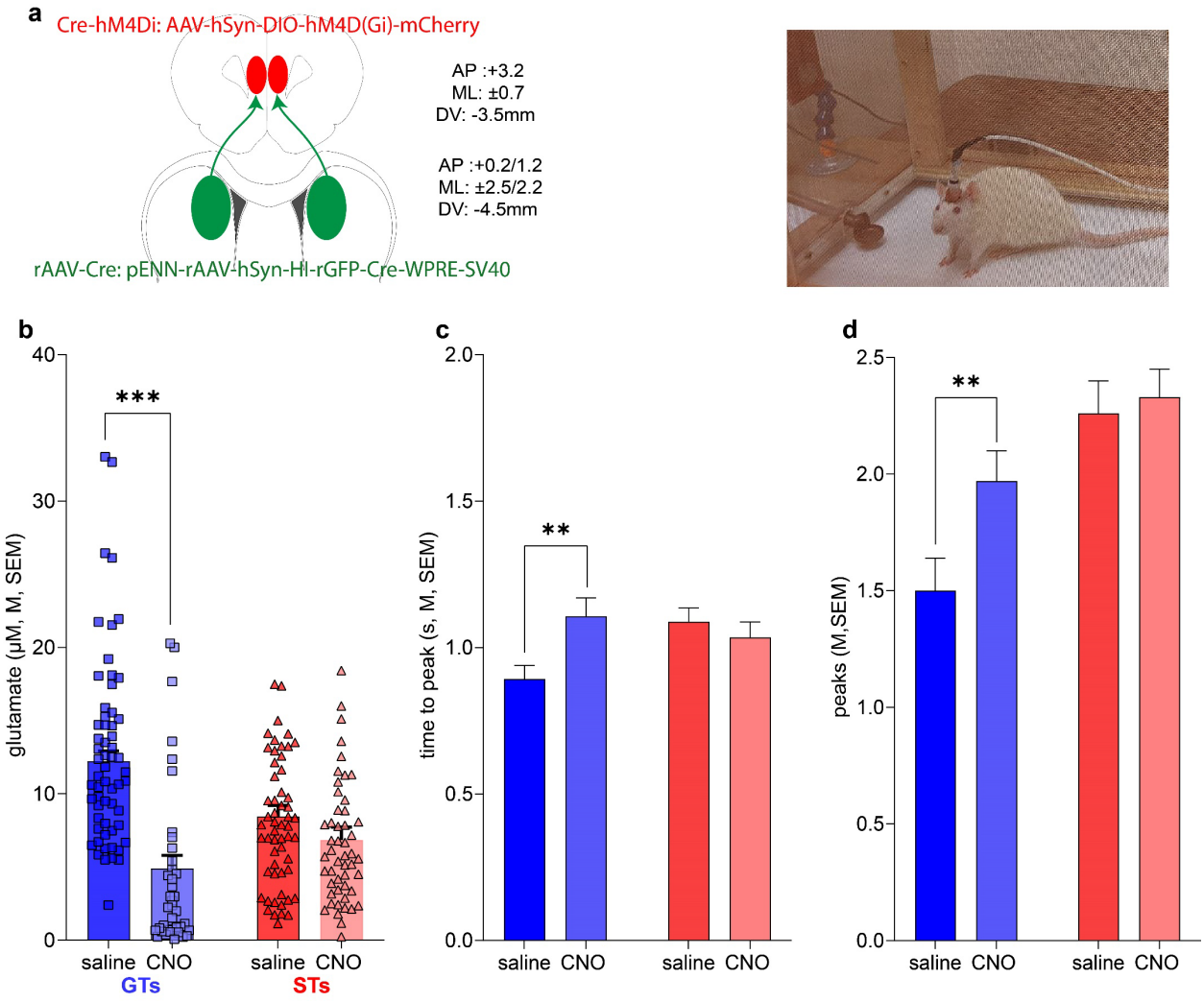


Figure 4.5: CNO administration attenuates GLU increases during cued turns in hM4Di GTs
a: Dual-vector scheme; an inhibitory Cre-dependent virus was bilaterally infused into the prelimbic cortex, while a retrograde Cre vector was bilaterally infused into dorsomedial striatum. b: GLU concentration increases (represented as EMM±SEM with individual values superimposed) during saline and peripherally injected CNO sessions. In CNO-treated, hM4Di-expressing GTs (purple) GLU levels were significantly lower when compared to levels in these same animals with vehicle (blue) and in hM4Di-expressing STs with vehicle (red) or CNO (pink) administered. Glutamate concentration in µM displayed on the y-axis. c: GLU increases in CNO-treated, hM4Di-expressing GTs were slower to peak (measured in seconds) and peaked more often (d) than increases in these same animals with saline injected and to increases from hM4Di-expressing STs with either injection. Post-hoc comparisons between injections within GTs: $p<0.001$***, $p<0.01$**.

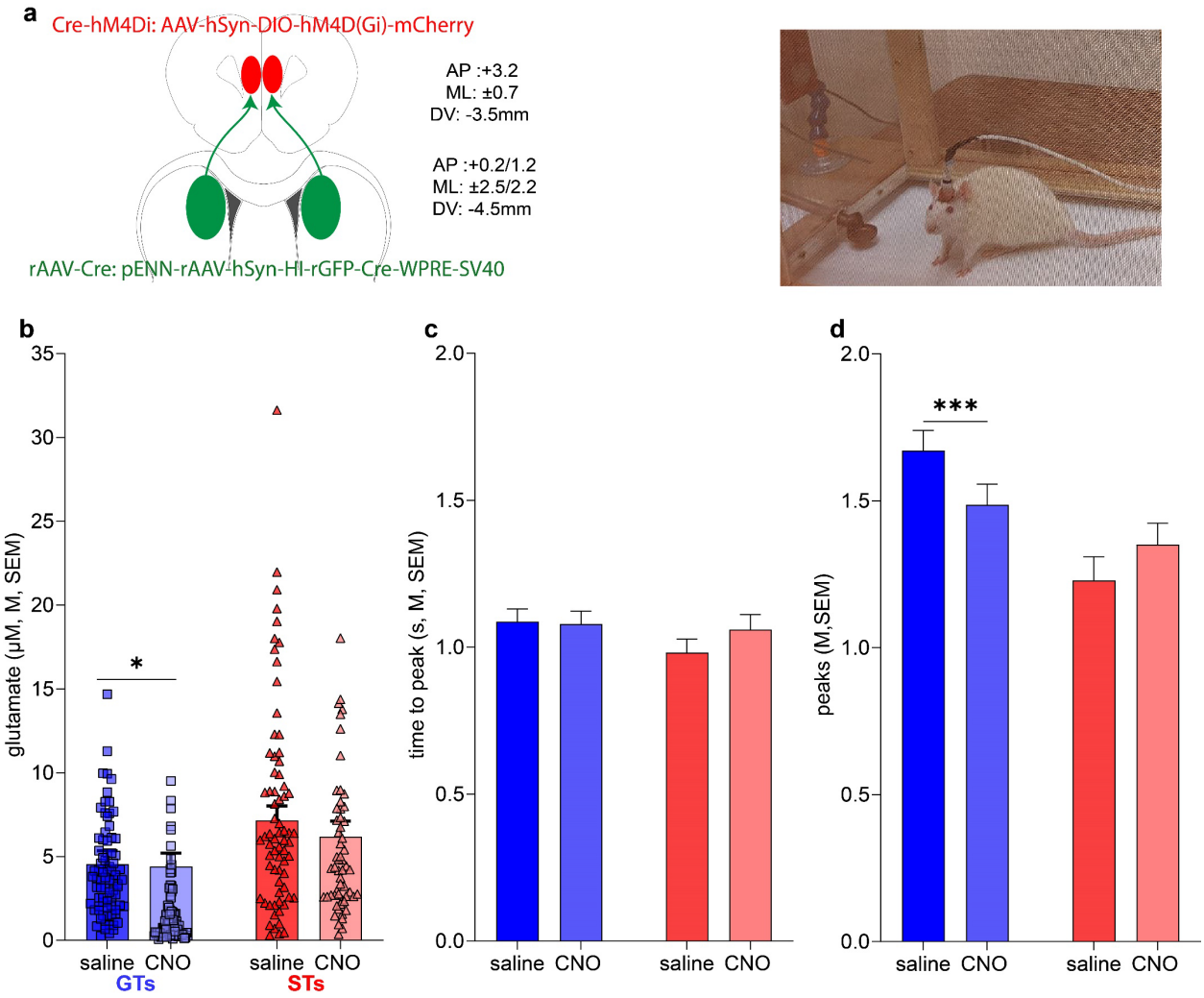


Figure 4.6: No effects of CNO administration on GLU in GTs or STs during cued stops

a: Dual-vector scheme; an inhibitory Cre-dependent virus was bilaterally infused into the prelimbic cortex, while a retrograde Cre vector was bilaterally infused into dorsomedial striatum. b: GLU increases (represented as EMM±SEM with individual values superimposed) during cued stops in hM4Di-expressing GTs with vehicle (blue) or CNO (purple) and hM4Di-expressing STs with vehicle (red) or CNO (pink) injected peripherally. GLU increases time-locked to the stop cue were unaffected by CNO administration. GLU increases in hM4Di-expressing GTs were significantly lower when compared to hM4Di-expressing STs with either injection. *above GTs indicates a main effect (p<0.05) of phenotype. c: CNO administration did not affect the time for GLU concentrations to peak or the total number of peaks (d) in hM4Di-expressing GTs or hM4Di-expressing STs during cued stops. GLU increases in vehicle- or CNO-treated hM4Di-expressing GTs peaked more than GLU increases from hM4Di-expressing STs with either injection. ***above GTs indicates a main effect (p<0.001) of phenotype.

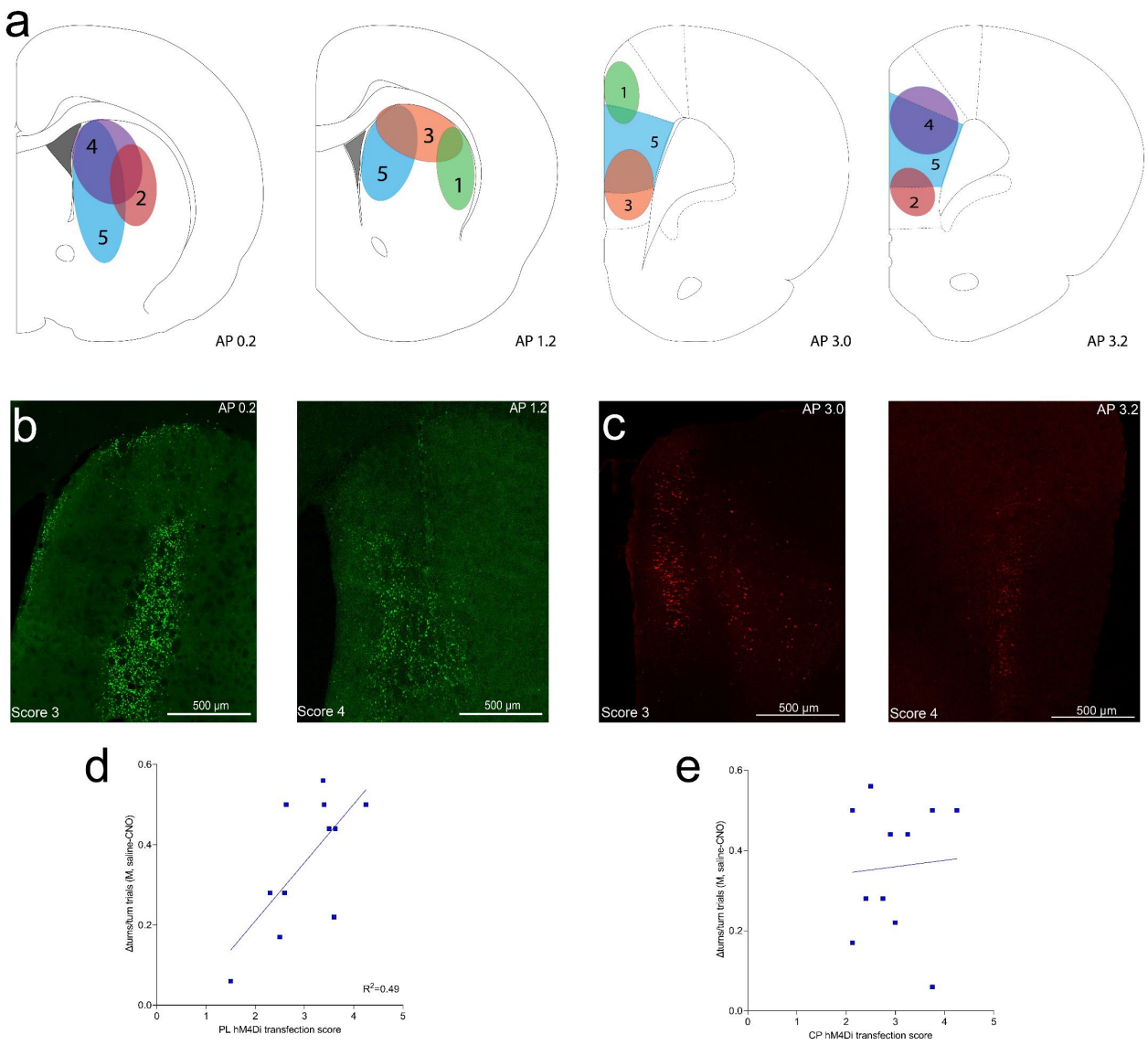


Figure 4.7: Visualization and transfection scores in the frontal cortex and striatum
a: Illustrations and representations of the transfection scoring scheme. On coronal sections, transfected areas that were restricted primarily to the prelimbic cortex and for the striatum, areas primarily restricted and located in the dorsomedial striatum were assigned the highest score (5), as opposed to areas that were more lateral or ventral were assigned scores (≤4). Representative images of coronal sections (10x) at AP 0.2 and 1.2 (b) and AP 3.0 and 3.2 (c). d: The sizes of the areas transfected in prelimbic cortex with the inhibitory DREADD construct, indicated by the transfection scores, were significantly correlated with GTs difference scores in turn rates (turn scores from CNO injected - saline injected CTTT sessions; R^2=0.49; P=0.02). e: No correlation was found between striatal transfection scores and difference scores in turn rates.

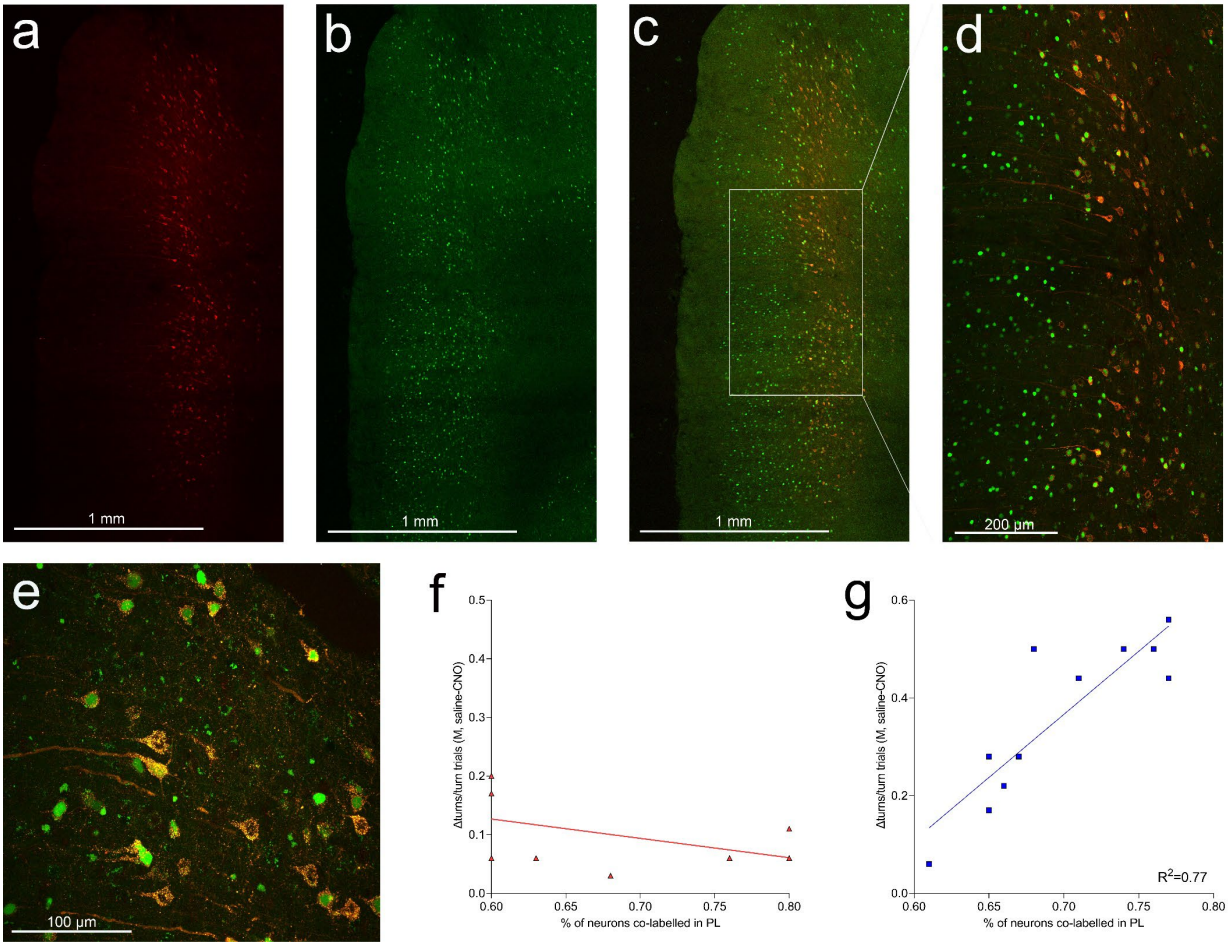


Figure 4.8: Co-localization of mCherry and GFP positive neurons
Coronal sections exemplifying mCherry-positive (DREADD reporter), GFP-positive (retrograde-Cre reporter), and double-labeled neurons in the frontal cortex (a-d; 1-mm scale inserted). e: Examples of double-labeled cells (100-µm scale inserted). Single- and double-labeled cells were counted in the transfection regions determined based on immunofluorescent stains (see Methods). f: The difference scores in turn rates for STs did not correlate with the percentage of co-labelled neurons. g: The percentage of neurons co-labelled was significantly correlated with the difference scores in GTs turn rates (turn scores from CNO injected - saline injected CTTT sessions; R^2=0.77; P=0.013).

Chapter V: General Discussion

Synopsis.

The work presented here addresses how environmental cues are imported into the dorsomedial striatum to integrate with ongoing movement to facilitate adaptive and purposeful motor shifts and corrections. *Chapter II* demonstrated that only cues preceding and predicting situations that require updating movement sequences (turns) elicited increases in dorsomedial glutamate (GLU) in GTs as opposed to stop-and-go sequences (stops). In contrast, GLU levels in STs were consistent across all trials and response. In *Chapter III* additional analyses are needed to refine the understanding of the behavioral impact of ITI variations in GTs. *Chapter IV* demonstrated that cue-triggered turning and cue-locked GLU increases in GTs depends on the activity of corticostriatal inputs. These results indicate that cued turning in GTs involves the integration of cue information onto ongoing movement sequences through fronto-striatal innervation. In contrast, animals with unresponsive cortical cholinergic signaling, STs, may rely primarily on striatal processes of cue-associated reward expectations for executing well-practiced turns. Collectively, these findings indicate distinct levels and patterns of dorsomedial striatal (DMS) GLU across phenotypes and behavioral responses mediated by differential engagement of overlapping circuitry. Each chapter's conclusions, limitations, and remaining questions are discussed below.

Evidence for cortical vs. subcortical engagement to perform the CTTT: GTs vs. STs.

In *Chapter II,* we asked if movement cues evoke glutamate release in the dorsomedial striatum. We leveraged animal models with opposing cognitive-motivational styles, characterized by responsive or unresponsive cortical cholinergic signaling- GTs and STs, respectively (Sarter & Phillips, 2018). We measured real-time GLU levels in the dorsomedial striatum (DMS) of these animal models performing a task involving turn and stop-and-go signals. Our observations revealed that turn cues resulting in turns elicited GLU increases in GTs, while in STs, GLU concentrations did not differ across responses.

Conversely, GLU concentrations in STs increased to a greater degree during reward delivery than in GTs. Previous studies found that cue detection relied on cholinergic activation of frontal cortices (Howe et al., 2017; McGaughy et al., 2002; Parikh et al., 2007). In this context, cue detection refers to the ability to process cues and respond according to established stimulus-response rules rather than just orienting to a task cue. These studies revealed that brief cholinergic signals, termed transients, triggered oscillations in the gamma frequency range and coordinated theta-gamma cross-frequency coupling. This indicated that these transients might facilitate the coordination of neuronal populations, transmitting information across cortical and subcortical regions to enable complex, cue-guided behaviors. Additionally, M1 muscarinic acetylcholine receptors, expressed on cortical interneurons and cortical output neurons, are necessary to generate these high-frequency oscillations and cross-frequency coupling (Howe et al., 2017). Furthermore, optogenetic activation of cortical cholinergic activity induced false reporting of signals, while inhibition increased the number of missed cues (Gritton et al., 2016).

Based on these findings, we hypothesized that movement cues might evoke dorsomedial glutamate due to cholinergic modulation of fronto-cortical inter- and output neurons, facilitating integration with striatal movement sequencing. GTs display greater complex movement control through timing forward movement to favorable/less challenging conditions and greater executive control in detecting rare signals over long times compared to STs (Kucinski et al., 2018; Paolone et al., 2013). This past work suggests greater engagement of cortical top-down regulation in GTs than in STs. As a result of GTs' propensity to engage top-down mechanisms, we predicted that the turn and stop cue in the CTTT would evoke glutamate in the dorsomedial striatum in GTs; however, results revealed that only turn cues resulting in successful turns evoked glutamate.

One key question from this finding is why do turns, but not stops, evoke GLU in GTs? The complexity of executing a turn versus a stop may account for this disparity. Turning requires reprogramming motor sequences by interrupting forward or redirecting movement from a pause. This process entails repositioning and coordinating limbs, maintaining balance during rotation, and potentially making corrective actions to offset errors. Similarly, patients with Parkinson's disease (PD) with a history of falls, thought to

reflect reduced cholinergic activity, commonly experience difficulties with turning (Chou & Lee, 2013; Grimbergen et al., 2004; Mancini et al., 2018; Müller & Bohnen, 2013; Naismith et al., 2010; Rochester et al., 2012). We also observed these turning but not stopping deficits in a rat model of Parkinsonian fallers (Avila et al., 2020).

Thus, the more complicated movement associated with a cued turn might depend on corticostriatal engagement to coordinate environmental cue processing and motor output successfully. In comparison, stopping and restarting in the same direction may not require updating and modification of movement sequencing and, therefore, not require fronto-cortical regulation. In this vein, in *Chapter IV*, silencing of hM4DI-expressing prelimbic inputs to the DMS via CNO administration significantly attenuated peak glutamate concentrations during cued turns but not stops. Silencing of these inputs also resulted in deficits in cue-triggered turning, but not stopping, in GTs.

However, this interpretation then raises the question of whether and how GTs utilize stop cues to accomplish the task (discussed in lingering questions and limitations). We also predicted that movement cues would either not evoke GLU increases in STs or would evoke truncated GLU amplitudes compared to GTs, reflecting the unresponsive cortical cholinergic systems of these animals. The amplitudes of GLU increases in during STs were lower than those seen in GTs, and, contrary to GTs, did not differ across responses. In *Chapter IV,* corticostriatal inhibition did not affect CTTT performance or glutamate concentrations in STs.

Altogether, these results indicate that cued turning in GTs involves top-down integration of cue information onto ongoing movement sequences through fronto-striatal innervation. In contrast, cued turning in animals with deficient cortical cholinergic signaling, STs appear to rely on less complex, primarily striatal processes for executing well-practiced turns. The potential mechanisms driving STs, performance are discussed below in the implications and limitations on interpretations of these findings.

Lingering questions and limitations.

How is the stop cue used to guide performance? During the development of the CTTT, we incorporated two modalities requiring animals to rely on external cues predictive of a reversal or restart of the belt to guide performance. One possibility is that animals learn to disregard the stop cue and/or rely on proprioception for stopping; however, given their

high levels of performance distinguishing between the turn and stop cue modalities during training and testing conditions, this seems unlikely. Another interpretation is that the stop cues inhibit a response, in this case turning, and thus, could be probing action impulsivity (Mayse et al., 2014; Morrow & Tomie, 2018). However, key differences exist between tasks commonly testing impulsive action, i.e. the choice reaction time task, the Go/No Go task, and the stop signal task, compared to the CTTT. These other tasks include measurements of premature responses and enforcing time-out periods. In the CTTT, animals must not turn during cue presentation to the restart period for a stop trial to be rewarded. We did not record premature turns, and time-out periods were not part of the design. Our *post hoc* video analyses of the total number of turns and rears did not reveal differences between STs and GTs in training conditions. They were not included in the work presented here. In the CTTT, STs and GTs were predominantly positioned in front of the reward port during stop trials, displaying similar low rates of false turns (~1.5 out of 18 total trials). Together, these results suggest that the stop cue does not solely test action impulsivity, and STs and GTs learn to discriminate between the cue modalities, indicating a stop or turn trial. One lingering question is if the rats process the turn and stop cues as separate prompts to two specific actions or, for example, preferentially process the turn cue as a prompt to turn and the stop cue as a non-turn cue and vice versa. Stop cues, as suggested in *Chapter IV,* do not require fronto-cortical involvement for GTs, specifically prelimbic cortical inputs to the dorsomedial striatum (DMS), and may be mediated by similar mechanisms used by STs to complete the CTTT (discussed below).

Reliance on bottom-up mechanisms in STs.

How do STs perform the CTTT, and why do STs display indiscriminate increases in dorsomedial GLU concentrations? One limitation of the work presented here is we tailored our manipulations to affect GTs' performance; therefore, the cognitive and neurobiological mechanisms by which STs perform are difficult to determine. As stated, the broadly receptive nature of GLU signaling in STs during the CTTT and lack of an effect of corticostriatal silencing (*Chapter IV)* imply that STs do not or cannot engage cortical top-down circuitry to perform the CTTT.

STs are proposed to exhibit a bias for stimulus-driven or 'bottom-up' attention reliant on perceptual and salience signal characteristics (Phillips & Sarter, 2020; Pitchers et al.,

2017a). Additionally, STs show greater activation in subcortical dopamine-mediated circuits, which are involved in initiating, motivated behavior (Flagel et al., 2011; Flagel & Robinson, 2017). Thus, STs may employ these subcortical circuits to perform the CTTT. To address if STs perform the CTTT through bottom-up attentional processes, we could manipulate the perceptual features of our cues. Animals perform the CTTT under red-light conditions to increase the visibility of the visual cue; however, manipulations of the luminance of the light cue and/or the decibels of the audio cue used for turn trials could test if STs rely on and are, therefore, vulnerable to perceptual changes. We could employ specific chemogenetic manipulations of subcortical dopamine-mediated and/or thalamic circuits to determine the role of these circuits in CTTT performance in sign-trackers.

What is the role of the corticostriatal pathway in GTs performing the CTTT?

Chapter II alluded to the differential engagement of fronto-cortico ciruitry of GTs and STs to perform the CTTT. *Chapter IV* determined that prelimbic cortical inputs are major contributors (if not the source) of GLU increases evoked by turn cues in GTs. Corticostriatal inhibition reduced amplitudes of GLU concentrations and decreased cue-triggered turns in GTs, while STs were unaffected. Importantly, transfection efficacy restricted to the prelimbic area, not the frontal cortex, correlated with the severity of turning deficits in GTs. Previous work recording cholinergic activity in the prelimbic cortex showcased that transient cholinergic activity was necessary for situations requiring attentional shifts, supporting this interpretation of corticostriatal engagement in demanding scenarios (Howe et al., 2013b). Additionally, stimulation of nicotinic acetylcholine receptors in the prefrontal cortex did not improve baseline SAT performance but improved performance in a version of SAT where a distractor is implemented (Howe et al., 2010).

In STs, the fast event rate resulting from shortening the ITI resulted in augmented GLU levels, possibly involving bottom-up mechanisms, such as dopaminergic modulation of glutamate linked to the increased reward rate; however, no effects of event rates were found on cued turns or stops in these animals. In contrast to GTs, increasing the proportion of turn cue presentations resulted in a higher number of cued turns in STs, without affecting GLU levels. The disconnected effects of task manipulations on the

performance of STs and dorsomedial GLU concentrations support that corticostriatal GLU does not drive STs' CTTT performance.

Lingering questions and limitations.

One potential limitation of the presented work is that we manipulate the inter-trial interval and vary the ratio of trial types to challenge GTs' performance by increasing attentional processing demands, as indicated in previous research (Parasuraman et al., 1987; Warm et al., 2008). *Chapter III* investigates the importance of glutamatergic signaling in GTs in CTTT performance. To challenge GTs' performance and examine associated GLU changes, we modified task parameters, including altering the inter-trial interval (ITI) and varying the proportion of trial types. These manipulations did not result in concurrent impacts on behavior and cue-locked GLU increases upon macro-analyses of overall cued turn and stop rates. However, dissociable effects on cue-triggered turning and GLU concentrations were observed. Shortening the ITI resulted in attenuated GLU amplitudes in GTs without affecting turn rats. In contrast, the short ITI condition for STs resulted in augmented GLU amplitudes, possibly involving bottom-up mechanisms (Lee et al., 2018). Extending the ITI increased cue-triggered turn rates in GTs without affecting cue-locked GLU concentrations. Additionally, decreasing the proportion of turn trials in the CTTT led to reductions in cued turns in GTs. Conversely, increasing the proportion of turn cue presentations resulted in more cued turns in STs. By conducting thorough analyses of various behavioral measurements within individuals and corresponding concentrations of glutamate across different conditions, we can potentially uncover the precise aspects that corticostriatal glutamate encodes. For example, future analyses could involve examining trial-to-trial variations in turn latencies alongside corresponding increases in glutamate levels to determine if there is a correlation between these factors may suggest modulation of glutamatergic activity potentially by dopamine or GABA (Corte et al., 2021; Cox & Witten, 2019). Investigating when errors within an individual are more likely to occur, such as after trials of the same modality or cross-modalities may have implications in attention and behavioral flexibility (Featherstone & McDonald, 2005; Ragozzino et al., 2002; Vibell et al., 2017; Zheng et al., 2021).

Another perspective to consider is that the corticostriatal pathway may also serve a role in learning and adapting to changes in action-outcome associations and related action

selection (Diesburg & Tatz, 2021; Hart et al., 2018; Morris et al., 2015; Rasooli et al., 2021). Further evidence supporting the role of GLU in encoding action-outcome associations showed that the blockade of glutamate receptors in the dorsomedial striatum, specifically NMDA receptors, prevents this learning (Yin et al., 2005a). These results may act in tandem, suggesting that corticostriatal glutamate is necessitated by dynamic situations requiring top-down control to match cognitive challenges such as changes to learned action-outcome associations. Additionally, corticostriatal inputs have been implicated in temporal processing to link these action-outcome associations (Emmons et al., 2019). Furthermore, ramp-like dorsomedial activity in rats was shown to persist between trials, with rapid switches in activity to signal outcome evaluations (Kim et al., 2013; Vandaele et al., 2021a). To address if corticostriatal GLU in the CTTT is important in encoding and/or signaling outcome changes to specific responses, recording from GTs after devaluing the reward or altering the contingency of whether a turn will lead to reward delivery after extensive training could determine the specific role of corticostriatal GLU in such behavioral adaptations.

An important lingering question from this work is how striatal GLU subsequently drives goal-directed action. The striatum holds a prominent position within the basal ganglia, characterized by its intricate web of connections and its involvement in a wide array of behaviors; therefore, dorsomedial glutamatergic release may interact with a multitude of receptors and cell types to guide behaviors (Hjorth et al., 2020; Oorschot, 1996). Cortical and thalamic projections release glutamate onto direct and indirect pathway GABAergic spiny output neurons “SPNs” and cholinergic interneurons “ChIs” (Hjorth et al., 2020). These parallel thalamostriatal projections, particularly the parafascicular thalamus, do not seem to be involved in initial learning, instead act in a modulatory capacity to facilitate corticostriatal updating action-outcome associations via regulating ChIs activity (Bradfield et al., 2013). Past work in our laboratory has indicated the importance of ChIs activity in cue-triggered turning in unscreened animals (Avila et al., 2020). Cortical innervation onto fast-spiking GABAergic interneurons may also provide forward inhibition to direct pathway SPNs. Thus, cortico-striatal activation may act in encoding response-outcome associations and adapting in conjunction with thalamic projections in dynamic scenarios through initiating and inhibiting action.

Valuable insights might be gleaned by using specific and temporally precise manipulations on direct and indirect SPNs during turn cue presentation in GTs. Specifically, insights into the underlying mechanisms by which striatal GLU signaling may govern cue-directed action selection, which cannot be entirely determined solely from the existing findings presented in this study.

Summary of completed work.

Chapter II examines the differences in dorsomedial striatal glutamate (GLU) increases evoked by movement cues in animal models exhibiting responsive (GTs) and unresponsive cortical cholinergic signaling (STs). In GTs, the presentation of turn cues, leading to successful turns, reliably elicited increases in GLU concentrations. In contrast, GLU levels in STs time-locked to task (turn and stop) cues were not distinguishable between specific behavioral responses. This indiscriminate GLU pattern in STs suggests potential bottom-up modulation through dopaminergic or thalamic inputs, likely driven by sensory stimuli presentation rather than linked to guide particular behaviors. The observation that increases in dorsomedial striatal GLU in GTs were exclusively triggered by turn cues that resulted in turns suggests a potential role of GLU signaling in guiding cue-directed action. *Chapter III* results hint at potential effects of shortening the ITI on dorsomedial GLU in GTs. However, additional in-depth and targeted analyses, may be needed to refine our understanding any behavioral impacts of these parameter manipulations on GTs. *Chapter IV* explores the origin of glutamate evoked by turn cues in GTs. Activating inhibitory DREADDs through CNO administration resulted in cue-triggered turning deficits specifically in GTs. Additionally, transfection efficacy in the prelimbic area correlated with the severity of deficits. Moreover, CNO administration in GTs reduced GLU (glutamate) transients. These CNO-modified transients shared similarities with transients in STs, characterized by the prolonged time to peak and an overall higher number of peaks. These results suggest that GTs rely on corticostriatal glutamatergic innervation for successful CTTT performance.

These findings carry substantial implications for various disease states. Take for instance, the case of Parkinson's disease patients prone to falls. As basal cholinergic terminal losses predict fall propensity, individuals indexed by goal-tracking behaviors may face increased risks for falls, as they are no longer able to rely on top-down cognitive

mechanisms and neural processes. In human sign-trackers, characterized by a weak top-down system these deficits may appear in earlier, and become apparent when challenged in unfamiliar, dynamic surfaces as they cannot engage cortical machinery to detect relevant cues, such as missteps and slips, to drive corticostriatal orchestration for movement reprogramming.

Concluding remarks.

Overall, the work presented here provides insights into the neural mechanisms and the significance of corticostriatal glutamatergic signaling underlying goal-directed action in complex movement control. Extensive work has implicated functional and structural abnormalities of frontostriatal circuitry in various disease states such as Parkinson's disease and disorders relating to reward processing (Aouizerate et al., 2004; Bohnen & Albin, 2011; Harada et al., 2021; Kerstetter et al., 2016; Lin et al., 2015; Makris et al., 2008; Orieux et al., 2002; Raschle et al., 2015). Moreover, cognitive-motivational styles have demonstrated to predict the severity and prognosis of psychiatric and neurological disorders (Anselme & Robinson, 2020; Boosman et al., 2012; Field & Cox, 2008; Kleiman & Riskind, 2012). Therefore, by examining the impact and interactions of cognitive-motivational styles on corticostriatal glutamatergic signaling, we may elucidate new avenues for understanding, diagnosing, and treating differing disease states, ultimately leading to more individually targeted and effective interventions.

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