Elucidating the Role of the Paraventricular Nucleus of the Thalamus and Cortico-Thalamic Circuitry in Cocaine-Seeking Behavior

by

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DEDICATION

This dissertation is dedicated to my parents, Joseph and Colleen, and siblings, Courtney, Bryan and Taylor for all their unconditional support throughout graduate school. I would also like to dedicate this dissertation to my fiancé, Joe, for his support and encouragement while finishing my degree.

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Abstract

Relapse continues to be one of the biggest problems in the treatment of addiction. This is due, at least in part, to the transformation of cues that are associated with the drug-taking experience into powerful motivators that can in turn elicit drug-seeking behaviors even when one desires to remain abstinent. However, the extent to which a cue can attain such motivational value varies between individuals, and only when it is attributed with incentive salience does it gain inordinate control over behavior. We use an animal model that allows us to study individual variation in the propensity to attribute incentive salience to reward-paired cues. In this model, sign-trackers (STs) are those rats that attribute incentive salience to a reward-predicting cue and will approach and manipulate the cue upon its presentation; whereas goal-trackers (GTs) assign only predictive value to the cue and go to the location of reward delivery upon cue presentation. Relative to GTs, STs are more susceptible to cocaine-primed and to cue-induced reinstatement of drug-seeking behavior. The paraventricular nucleus of the thalamus (PVT) is a brain region known to mediate individual differences in incentive salience attribution as well as drug-seeking behavior in various cocaine relapse models. In this dissertation, I present data showing that transient inactivation of the PVT prior to a test for cue-induced reinstatement selectively enhances drug-seeking behavior in GTs, without affecting behavior in STs. These data suggest that, in GTs, the PVT acts to attenuate the incentive motivational properties of a cocaine-cue during a test of cue-induced reinstatement. In a subsequent study I assessed the role of the cortical projections from

the prelimbic cortex (PrL) to the PVT in mediating cue-induced and cocaine-primed drug-seeking behavior. Inhibiting this pathway prior to a test for cue-induced reinstatement selectively decreases drug-seeking behavior in STs, without affecting behavior in GTs. However, drug-seeking behavior in either phenotype is not affected if this pathway is inhibited prior to cocaine-primed reinstatement. It appears, therefore, that the PrL-PVT circuit acts to enhance the incentive motivational value of a cocaine-cue selectively in STs, and does not mediate drug-seeking behavior to cocaine alone. Taken together, this work highlights the complex role of the PVT and its associated circuitry in mediating individual differences cue-reward learning and relapse propensity.

Chapter 1

Introduction

Note: Portions of the text, and both figures, within Chapter 1 have appeared previously in print (Kuhn et al., 2018, Maize Book), and are reproduced here with permission from the authors.

We are exposed to cues in our everyday lives that affect our decisions and actions. For example, if you are hungry and see a sign for a restaurant, you are more likely to go to that restaurant if your prior experiences there were enjoyable. This is just one example of how associative learning - establishing a relationship between a sign and a pleasurable experience - can guide our behavior. One type of associative learning strategy is called Pavlovian learning. During Pavlovian learning a once neutral stimulus reliably precedes the delivery of a reward (unconditioned stimulus, US). After being repeatedly paired with delivery of the reward-US, the neutral stimulus becomes a conditioned stimulus (CS), attains predictive value and elicits a conditioned response (CR) (Pavlov 1927). Pavlovian learning strategies are often advantageous, as they allow individuals to make associations between cues in the environment and resources necessary for survival, such as food and water. They also help individuals avoid dangerous or aversive situations, further promoting survival.

Conditioned stimuli, by definition, have predictive value, but they can also acquire incentive motivational value. That is, a CS can be transformed into an incentive stimulus or a "motivational magnet" (Berridge et al. 2009), by a process known as incentive salience attribution (Robinson et al. 1993, Berridge 2001). Incentive stimuli have three main properties: the ability to: 1) bias attention and elicit approach (e.g. Peterson et al. 1972, Harmer et al. 1998, Cardinal et al. 2002); 2) act as a conditioned reinforcer, in that animals will work for the presentation of the CS in the absence of the reward (e.g. Williams et al. 1991, Taylor et al. 1999, Di Ciano et al. 2004, Di Ciano et al. 2007); and 3) invigorate ongoing behavior during CS presentation (e.g. via Pavlovian to instrumental transfer) (e.g. Lovibond 1983, Dickinson et al. 2000, Wyvell et al. 2000). Importantly, whether a reward cue is attributed with predictive value or with both predictive and incentive motivational value, varies between individuals (Flagel et al. 2007, Flagel et al. 2008, Robinson et al. 2009). Individual differences in Pavlovian cue-motivated responses were first described in detail by Zener in 1937 (Zener 1937), not long after Pavlov's publication on "Conditioned Reflexes" (Pavlov 1927). In Zener's experimental paradigm, dogs were unharnessed and allowed to move relatively freely in response to presentation of a reward-paired CS, which in this case was a bell. For some dogs, exposure to this CS elicited approach to the site of reward delivery; while others, upon CS presentation, approached and interacted with the CS itself (Zener 1937). Decades later, CS-directed approach was termed "sign-tracking", as pigeons would follow or "track" the cue or "sign" that predicted reward delivery (Hearst et al. 1974). A few years later, using rats, Boakes (1977) coined the term "goal-tracking" based on his studies using rats that would approach the site of reward delivery upon cue-CS presentation and CS-elicited approach directed towards the CS itself as "sign-tracking" (Boakes 1977). Together, these studies highlighted the presence of individual variation

in Pavlovian cue-reward learning. More recently, Flagel and colleagues developed an animal model that captures this individual variation and allows us to dissociate the predictive value of a reward cue from the incentive motivational value (Flagel et al. 2007). Using this model, the neurobiological mechanisms underlying these two properties of reward-cues can be further dissected.

Individual variation in cue-reward learning

Using a Pavlovian conditioned approach (PCA) procedure (Flagel et al. 2007, Robinson and Flagel 2009, Meyer et al. 2012), rats can be characterized as goal-trackers (GTs), those that attribute predictive value to a reward-cue, or sign-trackers (STs), those that attribute predictive and incentive motivational value to the reward cue (Figure 1.1) During PCA training sessions, an illuminated lever (CS) enters the test chamber for 8 seconds, and upon its retraction a food pellet (US) is non-contingently dispensed into an adjacent food magazine. Rats that exhibit a goal-tracking CR go to the food magazine during lever-CS presentation; whereas rats that exhibit a sign-tracking CR approach and engage the lever-CS itself during presentation. Upon retraction of the lever-CS, all rats go to the food magazine to retrieve the food pellet that was delivered. While STs and GTs differ in their conditioned responses, both phenotypes learn their respective CRs at the same rate (Robinson and Flagel 2009).

The conditioned response of STs during PCA training aligns with the first property of an incentive stimulus, in that the lever-CS biases attention and elicits approach (Peterson et al. 1972, Harmer and Phillips 1998, Cardinal et al. 2002). The lever-CS is also a more effective conditioned reinforcer for STs relative to GTs. That is, for STs, the second property of an incentive stimulus is also met (Williams and Dunn 1991, Taylor and Horger 1999, Di Ciano and

Everitt 2004, Di Ciano et al. 2007). During a test of conditioned reinforcement, rats must poke their nose into a port for brief presentation of the lever-CS. However, delivery of the food-reward no longer follows lever-CS presentation. That is, the lever-CS is now the reinforcer. Thus, this test assesses the incentive motivational value of the lever-CS in the absence of the food reward. Compared to GTs, on a test of conditioned reinforcement, STs respond more for the presentation of the lever-CS, and press the lever more readily during its presentation (Robinson and Flagel 2009). As for the third property of an incentive stimulus, studies have not, in the classical sense, assessed Pavlovian-to-Instrumental Transfer (PIT) in STs and GTs. This is predominately due to methodological issues as a discrete localizable cue is needed to characterize rats as STs or GTs, as other cues (e.g. a tone) acts as a conditioned reinforcer equally in both phenotypes (Meyer et al. 2014). However, the use of a discrete localizable cue during Pavlovian training can then confound PIT training, as STs are likely to approach and engage with the Pavlovian cue, while GTs would not (Robinson et al. 2014). Yet, the ability of a reward-paired cue to acquire conditioned motivational properties and increase instrumental responding has been assessed. Rats were characterized as STs or GTs and then underwent cocaine self-administration training followed by extinction training. During extinction training, rats were non-contingently presented with the cue that was associated with cocaine infusions during self-administration training. STs showed greater drug-seeking behavior in response to this non-contingent cue presentation compared to GTs; and furthermore, the propensity to approach the lever-CS during PCA training correlated with cocaine-seeking behavior during this test (Saunders et al. 2013). Thus, rats that attribute incentive motivational value to a Pavlovian food-cue were more invigorated to seek drugs when presented with a cocaine-associated cue. In another study rats underwent cocaine-self administration and extinction training, followed by a

test for cocaine-primed reinstatement during which drug-seeking does not result in infusions of cocaine. However, rats were given an injection of cocaine prior to the test. The cocaine acted as an interoceptive cue associated with reward delivery during self-administration training and invigorated drug-seeking behavior to a greater extent in STs compared to GTs. (Saunders et al. 2011). These data suggest that cues associated with a reward can result in a conditioned motivational state that invigorates instrumental behavior and does so to a greater extent in rats that attribute incentive salience to a Pavlovian reward cue – that is, more so in STs than GTs.

Sign-tracking behavior is also persistent, as evident in Pavlovian extinction training and omission schedule training. During Pavlovian extinction training, whereby lever-CS presentations occur without subsequent food-reward delivery, STs decrease sign-tracking behavior at a slower rate compared to goal-tracking behavior in GTs (Ahrens et al. 2016). Interestingly, however, STs and GTs do not differ in the extinction of an instrumental response (Yager et al. 2010, Saunders and Robinson 2011, Saunders et al. 2014, Kawa et al. 2016, Kuhn et al. 2018). While there are many behavioral and neurobiological similarities between Pavlovian and instrumental extinction training, several differences are also present (for review see Todd et al. 2014) and likely contribute to this distinction. During omission training (i.e. lever deflections result in loss of food-reward delivery) rats decrease the number of lever deflections made, but still approach and interact with the lever without deflecting it (Chang et al. 2016). Thus, sign-tracking behavior (i.e. biasing attention and eliciting approach behavior) persists, despite training conditions changing. This type of persistence, or compulsive behavior, is not only characteristic of sign-tracking behavior, but also a central characteristic of drug addiction (for review see Tomie 1996, Kelley et al. 2002, Everitt et al. 2008, Berridge et al. 2016).

Individual variation in addiction-related behaviors

While attributing some motivational value to reward-cues can be adaptive, attributing excessive incentive motivational value to reward-cues can lead to maladaptive behaviors, such as drug addiction (Robinson and Berridge 1993, Berridge and Robinson 2016). For example, cues in the environment (e.g. people, places, paraphernalia) previously associated with the drug-taking experience can become incentive stimuli and gain inordinate control over behavior. Exposure to these stimuli, therefore, can elicit drug-seeking and drug-taking behavior and cause one to relapse in spite of the desire to remain abstinent (for review see Tomie et al. 2008). STs and GTs differ in several addiction-related behaviors. Relative to GTs, STs are more impulsive on tests of impulsive action (Flagel et al. 2010, Lovic et al. 2011). Additionally, relative to GTs, STs will sign-track to discrete cues associated with cocaine (Uslaner et al. 2006, Yager et al. 2013) and opioids (Yager et al. 2015), and work harder for delivery of cocaine (i.e. have a higher cocaine break-point) (Saunders and Robinson 2011). Although STs and GTs both readily acquire drug self-administration (Saunders et al. 2010, but see Beckmann et al. 2011, Saunders et al. 2013, Kawa et al. 2016, Kuhn et al. 2018) and do not differ in the rate of extinction of drug-seeking behavior (Saunders and Robinson 2011, Kawa et al. 2016, Kuhn et al. 2018), the two phenotypes do differ in drug-seeking behavior during tests for cocaine-primed and cue-induced reinstatement. During a test for cue-induced reinstatement, the action (e.g. nose poke) that previously resulted in the presentation of the drug and associated cue, now results in cue presentation without drug delivery. Thus, rats are responding based on the conditioned reinforcing properties of the drug-associated cue. During a test for drug-primed reinstatement, performing the action that previously resulted in drug delivery and cue presentation no longer results in either. However, prior to the start of the test rats are given an injection (or infusion) of

the drug. This test assesses the ability of the interoceptive properties of a drug to invigorate drug-seeking behavior. Compared to GTs, STs show greater cue-induced (Saunders and Robinson 2010, Saunders et al. 2013) and drug-primed (Saunders and Robinson 2011) drug-seeking behavior. Importantly, these differences between phenotypes have been shown following limited drug exposure (approximately two weeks), extinction training and, subsequently, a 2-4-week abstinence before the reinstatement test. We found, however, that when rats are exposed to a 2-week period of abstinence followed by extinction training and cue-induced reinstatement, the differences in cue-induced drug-seeking behavior between STs and GTs are not apparent (Kuhn et al. 2018). This suggests that the length and timing of the abstinence period with respect to extinction training is important for observing individual variation in cue-induced drug-seeking behavior. Additionally, if rats undergo prolonged cocaine self-administration training with intermittent access to cocaine, STs and GTs no longer show differences in several addiction-related behaviors, including drug-seeking behavior during tests of cocaine-primed and cue-induced behavior (Kawa et al. 2016).

Taken together, these data support the long-standing notion that Pavlovian incentive learning processes contribute to addiction-related behaviors (Bolles 1972, Bindra 1978, Toates 1981, Stewart et al. 1984, Robinson and Berridge 1993). By characterizing rats according to how they respond to Pavlovian reward-associated cues, we can predict which will be susceptible to relapse following relatively limited drug-taking experience, perhaps even before the development of compulsive drug-seeking behavior or the “transition to addiction” occurs (Vanderschuren et al. 2004, Belin et al. 2008, Robinson et al. 2008, Kasanetz et al. 2010, Piazza et al. 2013). Thus, by providing a means to parse the neurobiological mechanisms that mediate the predictive versus incentive motivational value of reward-cues, this model will help us

understand the neural processes that contribute to addiction-related behaviors and could lead to more effective and individualized treatments for addiction and the prevention of relapse.

Neurobiology of sign- and goal-tracking behavior

Research to-date has implicated the "motive circuit" as the primary network that differentially mediates sign- and goal-tracking behavior. The "motive circuit" is a set of cortical and subcortical nuclei that integrates information regarding a motivationally salient event, such as the presentation of a reward cue, and guide subsequent behaviors (Kalivas et al. 2005). Cortical structures, such as the prefrontal cortex (PFC), govern executive functions in the brain (for review see Fuster 2001, Jurado et al. 2007, Diamond 2013, Nyberg 2018). The PFC is believed to exert "inhibitory control," allowing one to attend only to the most meaningful stimuli and thereby mediate goal-directed behavior (for review see Ridderinkhof et al. 2004, Asplund et al. 2010, Mihindou et al. 2013). In contrast to cortical structures, subcortical structures tend to mediate aspects of emotions such as fear and reward (for review see Davis 1992, Baxter et al. 2002, Shin et al. 2010), autonomic functions such as hunger and sleep (for review see Salin-Pascual et al. 2001, Dietrich et al. 2013) and different forms of learning (for review see Baxter and Murray 2002, Liljeholm et al. 2012, Daniel et al. 2014). Nuclei throughout the cortical and subcortical components of the motive circuit communicate with one another to mediate various aspects of motivated behavior, ranging from encoding the value of the reward to determining the correct behavioral output to obtain that reward (for review see Kalivas and Volkow 2005). Thus, it is not surprising that dysregulation of this circuit contributes to addiction and relapse (Kalivas and Volkow 2005, Goldstein et al. 2011, Cerovic et al. 2013, Suckling et al. 2017).

STs and GTs differ in the extent to which they rely on the motive circuit. Relative to GTs, STs show greater engagement throughout this circuit in response to reward-paired cues (Flagel et al. 2011a, Yager et al. 2015) (Figure 1.2). To assess phenotype differences in cue-induced neuronal activity, rats were characterized as STs and GTs and then exposed to a lever-CS prior to sacrifice and quantification of c-fos as an index of neuronal activity. STs showed increased neuronal activity in the ventral and dorsal striatum, lateral septum, habenula, and several thalamic nuclei (Flagel et al. 2011a). Neuronal activation in GTs did not differ from an unpaired control group in any regions, including cortical regions (Flagel et al. 2011a). Greater activation of the motive circuit in STs relative to GTs also occurs in response to a drug-associated cue. In fact, there was considerable overlap between regions activated by a food- and opioid-associated cue in STs compared to GTs, including regions in the ventral and dorsal striatum, habenula and thalamus (Yager et al. 2015). Taken together, these data suggest that only when a reward cue is attributed with incentive salience (i.e. in STs) does it activate the motive circuit. Interestingly, when patterns of neuronal activity in response to a food-cue were examined between brain regions for a given phenotype, correlated activity was found between the cortical and subcortical areas for GTs, whereas for STs the correlated patterns of activity were restricted to subcortical regions (Flagel et al. 2011a). These data, as well as more recent findings (Flagel et al. 2017, Haight et al. 2017, Sarter et al. 2018), suggest that GTs rely on "top-down" cortical processes to inhibit the propensity to attribute incentive salience to reward cues; whereas enhanced "bottom-up" subcortical drive in STs increase that propensity (Figure 1.2).

"Top-down" cortical control

The PFC mediates top-down executive control in the brain, thereby maintaining goal-directed behaviors (Ridderinkhof et al. 2004, Asplund et al. 2010, Mihindou et al. 2013). That is, the PFC acts to selectively guide attention such that the focus is on the task at hand and not on "irrelevant" cues in the surrounding environment. As previously discussed, both phenotypes engage cortical regions in response to reward cues (Flagel et al. 2011a, Yager et al. 2015), but GTs specifically engage cortico-thalamic circuitry in response to a food-reward cue (Flagel et al. 2011a) (Figure 1.2). This suggests that goal-tracking behavior relies more on cortical control mechanisms to guide behavior. In fact, GTs are believed to have greater top-down attentional control than STs, and it has been postulated that a "deficit" in this top-down control contributes to the sign-tracking phenotype (for review see Sarter and Phillips 2018). In support, GTs perform better than STs on tasks that demand more cortical control, including those associated with impulse control (Flagel et al. 2010, Lovic et al. 2011) and sustained attention (Paolone et al. 2013).

PFC neurochemistry is also differentially affected in STs and GTs by the presentation of a Pavlovian cocaine cue. In STs, cocaine cue presentations elicit approach behavior and elevate dopamine levels in the PFC (Pitchers et al. 2017b). Interestingly, cue-elicited increases in dopamine levels correlate with higher levels of approach to the cocaine cue (Pitchers et al. 2017b), suggesting that cortical dopamine plays a role in encoding the incentive motivational value of the cue (but see also Ellwood et al. 2017). Conversely, in GTs, presentation of the cocaine cue does not elicit approach and does not affect dopamine levels, but does increase acetylcholine levels (ACh) (Pitchers et al. 2017b). Importantly, PFC ACh levels are not correlated with cue-elicited behaviors. These data highlight the involvement of distinct cortical processes in regulating the behavior of STs and GTs, and support the notion that GTs rely on

dopamine-independent cognitive processes to encode the meaning or value of reward cues (Dickinson et al. 2002, Flagel et al. 2011b, Sarter and Phillips 2018). That is, for GTs, as a function of enhanced cortical processing, a discrete reward cue is merely an "informational" stimulus that is relatively devoid of incentive properties (Flagel et al. 2011a). Thus, GTs exhibit goal-directed approach to the location of impending reward delivery if the reward is food, and explicitly do not approach drug-associated cues when no alternative behavioral response is available (i.e. when the drug reward is delivered intravenously).

"Bottom-up" subcortical control

Whereas goal-trackers are thought to rely primarily on "top-down" cortical mechanisms to guide their goal-directed behaviors, sign-trackers are believed to have enhanced "bottom-up" processing, as a function of increased activity in subcortical regions, including the striatum, amygdala, midline thalamus, and hypothalamus (Flagel et al. 2011a, Haight et al. 2017, Sarter and Phillips 2018). Moreover, cue-induced activity is correlated only between subcortical regions in sign-trackers, such that activity in midline thalamic nuclei correlates with neuronal activity in the ventral striatum (Flagel et al. 2011a, Haight et al. 2017) (Figure 1.2).

Striatum: Dopaminergic regulation of incentive motivational learning

Ventral Striatum

The nucleus accumbens (NAc), a region within the ventral striatum, is a key component of the motive circuit (Kalivas and Volkow 2005). The NAc receives dense dopaminergic projections from the ventral tegmental area, and this pathway, known as the mesolimbic pathway, plays an important role in reward-related processes (for review see Salamone et al.

2012, Volkow et al. 2017). Over the course of Pavlovian learning, STs and GTs show differences in the mesolimbic dopamine system, with emergent differences in gene expression (Flagel et al. 2007) and distinct patterns of phasic dopamine release in the nucleus accumbens (Flagel et al. 2011b). Phasic dopamine transmission in the NAc is known to be triggered initially by the receipt of a reward-US, but then, upon learning an association between a cue-CS and reward-US, the dopamine response shifts to the predictive-cue-CS (Schultz et al. 1997, Day et al. 2007). This pattern of dopamine activity is believed to support the "prediction error theory"; that is, that dopamine is acting primarily to encode the discrepancy between rewards received and those predicted (Montague et al. 1996, Waelti et al. 2001). Thus, an unpredicted reward initially elicits an increase in dopamine activity, or positive prediction error; a fully predicted reward elicits no response to the reward itself; and the omission of a predictive reward results in a decrease in dopamine activity, or negative prediction error (Schultz et al. 1997). The prediction error theory, therefore, suggests that dopamine is used to update the predictive value of stimuli during associative learning and thereby guide cue-elicited behaviors (Balleine et al. 2008).

In contrast to the prediction error theory, others have long postulated that dopamine acts to encode the incentive motivational value of reward cues (Berridge et al. 1998, Berridge 2007). Until the advent of the sign-tracker/goal-tracker model, however, it was difficult to parse the processes underlying predictive versus incentive learning, as the two were confounded in the majority of studies (for review see Robinson et al. 2014). Thus, the sign-tracker/goal-tracker model was exploited to address the long-standing debate in the field regarding the role of dopamine in reward learning. Using fast-scan cyclic voltammetry, which allowed the detection of dopamine on a sub-second time scale, Flagel, Clark and colleagues examined phasic dopamine release in the core subregion of the nucleus accumbens (NAcC) in response to cue and

reward presentation in STs and GTs during Pavlovian training (Flagel et al. 2011b). The NAcC was examined as it is considered a central locus for the dopamine-mediated effects of Pavlovian learning (Parkinson et al. 1999, Di Ciano et al. 2001, Parkinson et al. 2002, Dalley et al. 2005). Flagel, Clark, and colleagues found that the "classic" prediction-error shift in dopamine from the reward-US to the cue-CS occurs only in STs. That is, in GTs, the dopamine response does not differ between cue and reward presentation over the course of learning. Given that the reward cue (CS) is a predictor and elicits a conditioned response for both STs and GTs, these data demonstrate that the shift in phasic dopamine must be encoding the incentive value of the cue and not the predictive value (Flagel et al. 2011b). In support, when dopamine transmission is blocked via systemic administration of flupenthixol, a nonselective dopamine antagonist, the learning and expression of a sign-tracking response, but not goal-tracking, is attenuated (Flagel et al. 2011b). A subsequent study expanded upon these findings demonstrating specifically that dopamine in the NAcC is necessary for the expression of sign-tracking and not goal-tracking behavior (Saunders et al. 2012). Thus, sign-tracking is dopamine-dependent, and dopamine in the nucleus accumbens appears to be critical for incentive learning processes (Figure 1.2).

Dopamine transmission within the NAcC also plays an important role in individual variation in cue-induced reinstatement of drug-seeking behavior (Saunders et al. 2013). Relative to GTs, STs exhibit increased responding during a test for cue-induced reinstatement, and blockade of dopamine transmission in the NAcC significantly attenuates responding in STs, rendering them more like GTs (Saunders et al. 2013). In contrast, when dopamine concentrations in the NAcC are increased via administration of amphetamine, drug-seeking behavior increases in both STs and GTs (Saunders et al. 2013). Taken together, these data support a role for dopamine in encoding the incentive motivational value of reward cues and specifically suggest

that dopaminergic transmission within the NAcC is a critical part of the neurobiology underlying cue-induced drug-seeking behavior and the propensity to relapse.

Dorsal Striatum

The dorsal striatum, comprised of the caudate and putamen, is also known to play a role in motivation and addiction-related behaviors (Volkow et al. 2002, Balleine et al. 2007) and has been increasingly recognized for its role in habit formation (for review see Malvaez et al. 2018). Multiple subregions of the dorsal striatum are activated to a greater degree in sign-trackers relative to goal-trackers after presentation of a food- or drug-associated cue (Flagel et al. 2011a, Yager et al. 2015). One of these subregions, the dorsolateral striatum, has been investigated for its role in incentive salience attribution using the sign-tracker/goal-tracker animal model. When amphetamine is administered directly into this region, the conditioned response of both STs and GTs is amplified, and this is due to increased motivation, not habit formation (DiFeliceantonio et al. 2016). In contrast, however, neither blockade of dopamine signaling within the dorsolateral striatum nor inactivation of this region affects sign-tracking behavior (Fraser et al. 2017). This is true with the typical amount of training (i.e., 5 sessions), and persists after prolonged (i.e., 15 sessions) training, when the ventral striatum no longer mediates sign-tracking behavior (Clark et al. 2013). Thus, although enhanced dopamine signaling in the dorsolateral striatum can increase the incentive motivational value of a Pavlovian cue, making it a stronger motivational magnet, such incentive motivational processes appear to be dependent on dopamine signaling in the ventral and not the dorsal striatum (Flagel et al. 2011b, Saunders et al. 2013, Fraser and Janak 2017) (Figure 2.1).

Paraventricular Nucleus of the Thalamus

The paraventricular nucleus of the thalamus (PVT) is a midline thalamic nucleus located in an ideal position to influence motivated behaviors, as it acts as an interface to integrate cortical, emotion, and motor networks, and relays this information to the striatum (Kelley et al. 2005). Although this nucleus has been recognized as being part of the motive circuit for over a decade (Kelley et al. 2005), only recently has it gained attention in mediating several motivated behaviors such as stress (Bhatnagar et al. 1998, Bhatnagar et al. 2002, Li et al. 2010, Heydendael et al. 2011, Barson et al. 2015), fear responses (Li et al. 2014, Do-Monte et al. 2015, Penzo et al. 2015, Chen et al. 2018), and feeding behaviors (for review see Millan et al. 2017). Several addiction-related behaviors also engage the PVT. Administration of psychostimulants increases c-fos expression in the PVT (Deutch et al. 1995, Deutch et al. 1998, Stephenson et al. 1999), and alcohol exposure alters levels of neuropeptides within the PVT (Pandey et al. 2017, Gupta et al. 2018). Inhibiting neuronal firing within the PVT decreases cocaine conditioned place preference (Browning et al. 2014) as well as context-induced (Hamlin et al. 2009), cocaine-primed (James et al. 2010), and cue-induced reinstatement of drug-seeking behavior (Matzeu et al. 2015, Matzeu et al. 2016). The PVT and associated circuitry have also been shown to play a role in different stages of addiction such as drug-taking (Neumann et al. 2016) and drug-seeking behavior (Hamlin et al. 2009, Giannotti et al. 2018), as well as withdrawal (Zhu et al. 2016).

Prior studies suggest that the PVT acts as a central node to mediate sign-tracking and goal-tracking behavior. This region shows an increase in neuronal activation in STs compared to GTs in response to a food- (Flagel et al. 2011a) or drug-associated cue (Yager et al. 2015) (Figure 1.2). Yet, lesions to the PVT made prior to Pavlovian training amplifies sign-tracking behavior and attenuates goal-tracking behavior (Haight et al. 2015). When the lesion is made after rats have acquired their conditioned response, the behavior of sign-trackers is not affected

(likely due to a ceiling effect); but in GTs, goal-tracking behavior is decreased and sign-tracking behavior increased (Haight et al. 2015). These data suggest that the PVT may be acting as a "brake" on the attribution of incentive salience to reward cues. Thus, when the PVT is "off-line," the incentive motivational value of a reward cue is enhanced.

PVT Circuitry

It is evident that the PVT plays a pivotal role in mediating motivated behaviors, specifically those that are cue-motivated; however, the PVT circuitry implicated in these behaviors has been largely unexplored. The PVT is a common locus that differentially mediates cue-induced responsivity in STs and GTs (Flagel et al. 2011a, Haight et al. 2014). To further explore the PVT-circuitry that might be differentially regulating the behavior of sign- and goal-trackers, cue-induced neuronal activity selectively in neurons that were directly communicating with the PVT was examined (Haight et al. 2017). Relative to controls, both STs and GTs exhibit enhanced cue-induced activity in neurons in the prelimbic cortex that project to the PVT. In contrast, however, STs exhibit enhanced cue-induced activity in subcortical areas, including neurons from the lateral hypothalamus and medial amygdala that project to the PVT, and neurons in the PVT that project to the nucleus accumbens (Haight et al. 2017) (Figure 1.2). These findings will be further discussed in the following sections, but nonetheless support the notion that enhanced "bottom-up" processing largely contributes to the sign-tracking phenotype, whereas "top-down" processing contributes to goal-tracking behavior.

PVT Circuitry: Cortical Connections

Cortical projections to the PVT have been shown to mediate appetitive associative reward learning, as excitation of the PFC-PVT pathway decreases the acquisition and expression of

reward seeking in response to a conditioned stimulus (Otis et al. 2017). One region of the medial PFC, the prelimbic cortex (PrL), has been implicated in mediating both drug- and cue-motivated behaviors (Di Pietro et al. 2006, Di Ciano et al. 2007, Moorman et al. 2015). The PrL sends the densest set of glutamatergic projections *to* the PVT, while receiving reciprocal glutamatergic projections *from* the PVT (Li et al. 2012). This projection has been shown to contribute to fear learning in response to an aversive cue (Do-Monte et al. 2015), drug-seeking behavior (Giannotti et al. 2018), and Pavlovian cue-reward associations (Haight et al. 2017). Although cue-induced neuronal activity does not differ between STs and GTs in the PrL (Flagel et al. 2011a, Yager et al. 2015), correlated activity between the PrL and PVT is evident only in GTs, suggesting that this structure might play a role in differentially mediating the behavioral phenotypes (Flagel et al. 2011a, Haight and Flagel 2014). However, in response to a food-associated cue, STs and GTs engage projections from the PrL to the PVT to the same degree (Haight et al. 2017). Thus, since the reward cue is a predictor (i.e. it elicits a conditioned response) for both STs and GTs, it is likely that the PrL-PVT circuit encodes the predictive qualities of the cue-CS and may play an important role in exerting cognitive control in GTs (Figure 1.2). In STs, enhanced subcortical activity is likely overriding this cortical activity and driving the incentive motivational processes.

PVT Circuitry: Subcortical Connections

STs and GTs differ in a number of subcortical brain regions that project to the PVT, including subnuclei of the amygdala and multiple subregions of the hypothalamus. Medial amygdala (MeA) neurons projecting to the PVT showed greater cue-induced neuronal activity in STs relative to controls (Haight et al. 2017) (Figure 1.2). While little is known about the role of the MeA in appetitive-motivated behaviors, early work demonstrated that rats will bar press for electrical stimulation, or self-stimulate the MeA, suggesting that this nucleus does indeed play a

role in reward processing (Kane et al. 1991). However, additional work is needed to elucidate the function of the MeA in the circuits that appear to be mediating incentive motivational learning.

Neurons in the lateral hypothalamus (LH) that project to the PVT also show greater cue-induced activity in STs relative to GTs and controls (Haight et al. 2017) (Figure 1.2). The hypothalamus is known to play an important role in the motive circuit, as it is composed of multiple subregions with various key functions (Kelley et al. 2005). While the dorsomedial nucleus regulates autonomic functions such as blood pressure; the LH mediates aspects of motivation, state-dependent arousal, learning and feeding behaviors (for review see Stuber et al. 2016, Tyree et al. 2017). Thus, it is not surprising that the LH may play an important role in incentive motivational processes. The LH sends orexinergic projections to the PVT (Kirouac et al. 2005, Lee et al. 2015, Lee et al. 2016), and the role of PVT orexin signaling in addiction-related behaviors has gained increasing attention in recent years (James et al. 2011, Yeoh et al. 2014, Matzeu et al. 2016). For example, administration of orexin into the PVT invigorates drug-seeking behavior, while blockade of orexin signaling in the PVT prevents cue-induced cocaine-seeking behavior (Matzeu et al. 2016). We have found that antagonism of orexin receptors in the PVT attenuates the incentive motivational value of a reward cue and decreases sign-tracking behavior (Haight 2016, Campus et al. 2017). Thus, orexin signaling in the PVT may be a critical component of the neurobiological mechanisms underlying incentive salience attribution.

In addition to examining patterns of cue-induced neuronal activity in regions that send projections to the PVT, we were interested in examining differences in activity in neurons projecting from the PVT to the ventral striatum, a region we know is key in modulating individual differences in reward learning (Flagel et al. 2011b, Saunders et al. 2013). As expected, we found that, relative to controls, STs show enhanced cue-induced activity in neurons

projecting from the posterior PVT to the NAc (Haight et al. 2017) (Figure 1.2). Importantly, the NAc is a main target of PVT projections, with the most dense projections going to the shell of the NAc (Dong et al. 2017). The PVT can independently elicit dopamine release within the NAc shell as well (Parsons et al. 2007). Activation of anterior PVT projections to the NAc (predominately shell) attenuates cue-induced sucrose-seeking behavior, whereas inhibition increases sucrose-seeking behavior (Do-Monte et al. 2017). Neither of these manipulations affect general locomotor activity, anxiety-related behavior, or consumption of food reward, suggesting this circuit is specific to mediating cue-motivated behaviors (Do-Monte et al. 2017, but see Cheng et al. 2018). Furthermore, the PVT to the NAc shell pathway has been implicated in several addiction-related behaviors, including context-induced reinstatement (Hamlin et al. 2009), long-term effects of cocaine (Neumann et al. 2016), and opiate dependence (Zhu et al. 2016).

Taken together, the PVT seems to act as a hub that integrates cortical and subcortical information to guide behavior, but it does so to varying degrees in sign- and goal-trackers. Work thus far suggests that STs rely on enhanced hypothalamic-thalamic-striatal circuitry, whereas the behavior of GTs is primarily mediated by cortical-thalamic processes. Our working hypothesis is that the subcortical processes in STs override the cortical control mechanisms, permitting the attribution of incentive salience to reward cues in an excessive manner.

Conclusion

The sign-tracker/ goal-tracker model not only captures individual variation in the propensity to attribute incentive motivational value to reward cues but also individual variation in addiction-related behaviors, such as relapse propensity, following relatively limited drug-

taking experience. Using this model, we are able to dissociate the predictive from the incentive value of reward cues and explore the neurobiological mechanisms underlying these distinct associative learning strategies. The PVT appears to act as a central node mediating sign- and goal-tracking behavior, however the extent to which the PVT exerts control over individual variation in cue-induced drug-seeking behavior remains unknown. To date, we know that the behavior of sign-trackers is dopamine-dependent and seemingly reliant on subcortical hypothalamic-thalamic (PVT)-striatal pathways; whereas that of goal-trackers is dependent on cortical cognitive processes, including cortical-thalamic (PVT) processes. Taken together, we believe it is the imbalance between "top-down" versus "bottom-up" processing that drives the extreme behaviors inherent to each of the phenotypes, including deficits in attentional processing, impulsive behavior and increased propensity to relapse that are characteristic of sign-trackers. The role of the PVT and its circuitry in mediating individual variation in drug-seeking behavior will be addressed in this dissertation.

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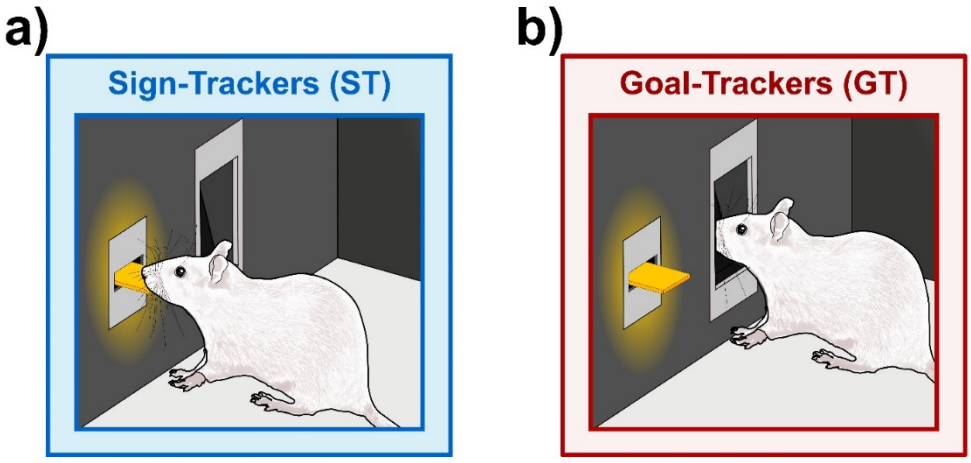

Fig 1.1 Pavlovian conditioned approach (PCA) training. Rats undergo Pavlovian conditioned approach training whereby an illuminated lever (conditioned stimulus, CS) is inserted into the testing chamber for 8 seconds and immediately upon its retraction a food reward (unconditioned stimulus, US) is delivered to the food cup. At the conclusion of training, rats are characterized as sign-trackers (STs) or goal-trackers (GTs). a) STs are those who are attracted to, manipulate and engage with the lever-CS during its presentation; whereas b) GTs are those who upon lever-CS presentation orient toward the CS, but then immediately go to the food cup to await reward delivery. While the lever-CS is a predictor and elicits a conditioned response for both STs and GTs, only for STs is it also attributed with incentive value and thereby transformed into a "motivational magnet."

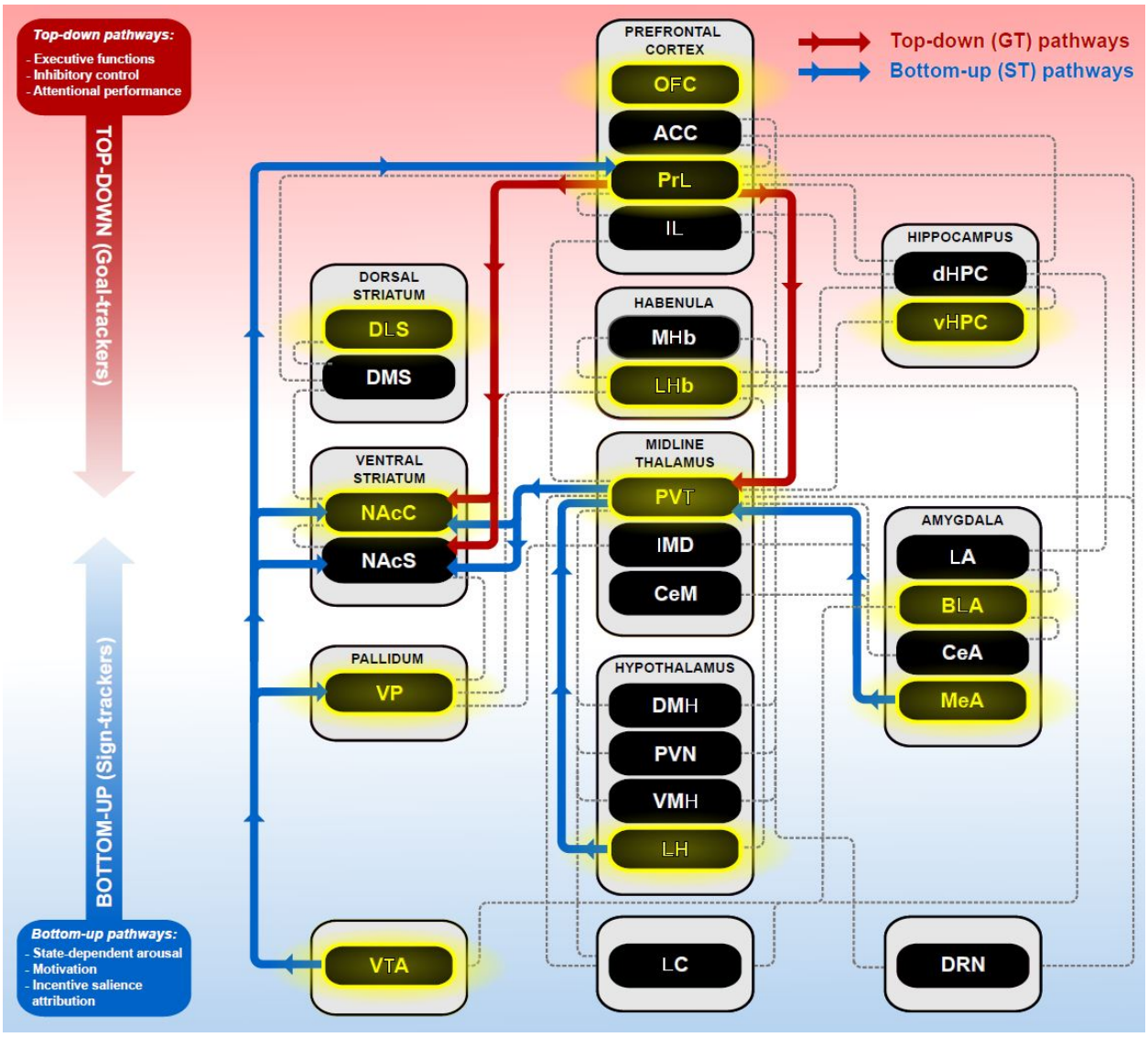

Fig 1.2 A schematic diagram of the brain circuitry involved in mediating individual variation in cue-motivated behaviors. All of the brain regions listed have been identified as part of the "reward" or "motive" circuits of the brain, and those highlighted in yellow have specifically been investigated for their role in incentive salience attribution. Dotted lines indicate known connections between brain areas and solid lines are proposed circuits regulating the behavior of goal-trackers (red) and sign-trackers (blue). The behavior of goal-trackers is thought to be mediated via "top-down" cognitive control processes; whereas that of sign-trackers is mediated via "bottom-up" subcortical processes. As indicated in the main text, we believe that it is an imbalance between the "top-down" versus "bottom-up" processes that contributes to each of the extreme phenotypes. Abbreviations: ACC, anterior cingulate cortex; BLA, basolateral amygdala; CeA, central nucleus of the amygdala; CeM, central medial nucleus of the thalamus; dHPC, dorsal hippocampus; DLS, dorsolateral striatum; DMH, dorsomedial nucleus of the hypothalamus; DMS, dorsomedial striatum; DRN, dorsal raphe nucleus; IL, infralimbic cortex; IMD, intermediodorsal nucleus of the thalamus; LA, lateral amygdala; LH, lateral hypothalamus;

LHb, lateral habenula; LC, locus coeruleus; MeA, medial nucleus of the amygdala; MHb, medial habenula; NAcC, core of the nucleus accumbens; NAcS, shell of the nucleus accumbens; PrL, prelimbic cortex; PVN, paraventricular nucleus of the hypothalamus; PVT, paraventricular nucleus of the thalamus; VMH, ventromedial nucleus of the hypothalamus; vHPC, ventral hippocampus; VP, ventral pallidum; VTA, ventral tegmental area.

Chapter 2

Transient inactivation of the paraventricular nucleus of the thalamus enhances cue-induced reinstatement in goal-trackers, but not sign-trackers

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Abstract

The PVT has been shown to mediate cue-motivated behaviors, such as sign- and goal-tracking, as well as reinstatement of drug-seeking behavior. However, the role of the PVT in mediating individual variation in cue-induced drug-seeking behavior remains unknown. Thus, the objective of Chapter 2 is to determine if inactivation of the PVT differentially mediates cue-induced drug-seeking behavior in sign-trackers and goal-trackers. To accomplish this, rats were characterized as sign-trackers or goal-trackers based on their Pavlovian conditioned approach behavior. Rats were then exposed to 15 days of cocaine self-administration, followed by a 2-week forced abstinence period and then extinction training. Rats subsequently underwent tests for cue-induced reinstatement and general locomotor activity, prior to which they received an infusion of either saline (control) or baclofen/ muscimol (B/M) to inactivate the PVT. Inactivation of the PVT selectively enhanced cue-induced drug-seeking behavior in goal-trackers; without affecting the behavior of sign-trackers. These findings further support the

notion that the PVT acts to differentially regulate cue-motivated behaviors in goal-trackers and sign-trackers.

Introduction

For addicted individuals, relapse often results from exposure to cues (e.g. people, places, paraphernalia) that have been associated with the drug-taking experience (for review see Shaham et al. 2003, Tomie et al. 2008). Exposure to these cues alone can cause intense feelings of craving (Childress et al. 1988, Childress et al. 1993), which can, in turn, elicit drug-seeking behaviors (see Shaham et al. 2003). These cue-reward associations are, in part, mediated by Pavlovian learning processes. During Pavlovian learning, a cue that reliably precedes the delivery of reward acquires predictive value. That is, the cue becomes a predictor, signaling the availability of reward. However, predictive cues can also acquire incentive motivational value, rendering them into powerful motivators and making them desirable in-and-of themselves (Stewart et al. 1984, Robinson and Berridge 1993). This process, known as incentive salience attribution, transforms predictive stimuli into "motivational magnets" (Berridge et al. 2009), allowing these stimuli to gain inordinate control and elicit maladaptive behaviors, such as compulsive drug seeking. Importantly, only for some individuals do reward cues acquire both predictive and incentive properties.

Using a Pavlovian conditioned approach (PCA) paradigm, we have shown that rats can be classified as goal-trackers (GTs), those that attribute reward-cues primarily with predictive value, or sign-trackers (STs), those that attribute both predictive and incentive value to reward-cues. In this paradigm, the presentation of a lever (conditioned stimulus, CS) always precedes the delivery of a food reward (unconditioned stimulus, US). That is, food delivery is non-contingent upon an instrumental response. While both GTs and STs learn the relationship between the lever-

CS and food-US, the nature of their Pavlovian conditioned approach response differs. Upon lever-CS presentation, rats classified as GTs attend to the location of impending food delivery; while STs approach and manipulate the lever-CS itself. Relative to GTs, STs also respond more avidly for presentation of the lever-CS during a test of conditioned reinforcement (Robinson and Flagel 2009). The ability of the lever-CS to bias attention and elicit approach behavior, and to acquire reinforcing properties (Robinson and Flagel 2009), indicates that the reward-cue has become imbued with incentive value for STs, to a greater extent than GTs. This enhanced propensity to attribute incentive salience to food-cues has been associated with a number of other addiction-related behaviors. For example, rats that sign-track to food-associated cues do the same to cues associated with drugs of abuse, including cocaine and opioids (Yager and Robinson 2013, Yager et al. 2015). In addition, relative to GTs, STs are more impulsive (Flagel et al. 2010, Lovic et al. 2011), have higher cocaine break-points (Saunders and Robinson 2011), and are more susceptible to cue-induced reinstatement of drug-seeking behavior (Saunders and Robinson 2010, Saunders et al. 2013, see also Kawa et al. 2016). Thus, the sign-tracker/goal-tracker animal model supports the long-standing notion that Pavlovian incentive learning processes are critical to drug-motivated behaviors (Bolles 1972, Bindra 1978, Toates 1981, Stewart et al. 1984, Robinson and Berridge 1993).

The sign-tracker/goal-tracker animal model has provided a novel foundation to dissociate the neural mechanisms underlying predictive vs. incentive learning (Flagel and Robinson 2017). Indeed, using this model, it has been shown that food- and drug-associated cues engage different circuitry in STs vs. GTs (Flagel et al. 2011a, Yager et al. 2015, Haight et al. 2017). Relative to GTs, STs show greater engagement of the so-called “motive circuit” (Kalivas and Volkow 2005), suggesting that this circuit encodes the incentive properties of reward cues (Flagel et al.

2011a, Haight and Flagel 2014). One brain region showing robust ST/GT differences in cue-induced neuronal activation is the paraventricular nucleus of the thalamus (PVT) (Flagel et al. 2011a, Yager et al. 2015). The PVT is a midline thalamic structure that acts as an interface between cortical, limbic and motor circuits, relaying information regarding arousal and reward, among other functions, to the striatum (Kelley et al. 2005). Thus, it is not surprising that this nucleus has been implicated in reward learning (Flagel et al. 2011a, Haight et al. 2015, Yager et al. 2015, Do-Monte et al. 2017, Haight et al. 2017, Ong et al. 2017, Otis et al. 2017) as well as a number of other complex behaviors, including fear learning (Li et al. 2014, Do-Monte et al. 2015, Penzo et al. 2015) and anxiety-related behaviors (Li et al. 2010, Barson and Leibowitz 2015). Work from our laboratory suggests that the PVT acts as a central node via the hypothalamic-thalamic-striatal axis to regulate the attribution of incentive salience to reward cues and the expression of the resultant behaviors (Haight et al. 2017). Using excitotoxic lesions, we have shown that taking the PVT "offline" causes an increase in sign-tracking behavior to a food-paired cue in rats with an inherent tendency to goal-track (Haight et al. 2015). Thus, the PVT appears to act as a "brake" on incentive motivational processes, and releasing this brake allows for the attribution of incentive salience to reward cues and/or expression of corresponding cue-motivated behaviors, at least in goal-trackers.

In recent years, the PVT has been increasingly acknowledged for its role in addiction-related behaviors (Deutch et al. 1995, Deutch et al. 1998, Young et al. 1998, Stephenson et al. 1999, James et al. 2013, Browning et al. 2014, Haight and Flagel 2014, Yeoh et al. 2014, Neumann et al. 2016, Zhu et al. 2016, Matzeu et al. 2017), with a particular emphasis on reinstatement of drug-seeking behavior (Hamlin et al. 2009, James et al. 2010, Matzeu et al. 2015, Matzeu et al. 2016). However, these prior studies were not designed to examine individual

differences in the role of the PVT in cue-motivated behaviors (Flagel et al. 2011a, Haight and Flagel 2014, Yager et al. 2015, Haight et al. 2017). In the current study, we assessed whether the role of the PVT in cue-induced drug-seeking behavior differs depending on inherent individual differences in cue-reward learning. To do so, rats were first exposed to Pavlovian conditioning and characterized as STs or GTs, and subsequently underwent 15 days of cocaine self-administration followed by 2 weeks of forced abstinence. Following extinction training, rats were tested for cue-induced reinstatement of drug-seeking behavior, prior to which rats received an infusion of either saline or a cocktail of baclofen and muscimol ($GABA_B$ and $GABA_A$ agonists, respectively) to transiently inactivate the PVT. Based on our prior work demonstrating that a lesion to the PVT enhances the incentive motivational value of a reward cue selectively in GTs (Haight et al. 2015), we hypothesized that inactivating the PVT would result in an increase in cue-induced cocaine-seeking behavior in GTs, rendering them comparable to STs. That is, removal of the PVT "brake" in GTs would result in the expression of incentive value of the cocaine-cue and thereby enhance cue-induced cocaine-seeking behavior selectively in this phenotype.

Methods

Subjects

A total of 252 male Sprague-Dawley rats weighing between 200-250 g upon arrival from Charles River (Saint-Constant, Canada and Raleigh, NC, USA) were initially screened for use in this study. Upon arrival, rats were pair-housed in a climate-controlled room with a 12-hour light: dark cycle (lights on at 06:00 h or 07:00 h depending on daylight savings time). Rats had ad libitum access to water and food throughout the entire study. Rats were allowed to acclimate to

the new environment for seven days before the experiment began. After surgeries, all rats were single housed for the remainder of the study to decrease the chance of damage to the surgical implants. All behavioral testing occurred during the light cycle, between 08:00 h to 19:00 h. Testing times for specific procedures are included below. The experimental timeline is shown in Fig. 2.1, with details of each procedure in the following sections. All experimental procedures conformed to the standards in *The Guide for the Care and Use of Laboratory Animals: Eight Edition*, revised in 2011, published by the National Academy of Sciences, and approved by the University of Michigan Institutional Animal Care and Use Committee.

Pavlovian conditioned approach (PCA) training

After the 7-day acclimation period, rats were handled for three days and given 45-mg banana-flavored grain pellets (about 30 pellets per cage; Bio-Serv, Flemington, NJ, USA) in their home cage. This allowed the rats to habituate to the experimenters as well as the food reward used during Pavlovian conditioned approach (PCA) training. PCA training occurred in standard behavioral testing chambers (MED Associates, St. Albans, VT, USA; 20.5 x 24.1 cm floor area, 29.2 cm high) housed within sound-attenuating boxes equipped with a ventilation fan to provide air circulation and constant background noise. In the center of one of the walls of the testing chamber was a food magazine located 6-cm above the grid floor and attached to a pellet dispenser. The food magazine was equipped with an infrared photobeam that, when broken, recorded “contact” with the food magazine. To the right or the left of the food magazine, and at the same height, was a retractable lever that was illuminated upon presentation. A minimum of 10-g of force was necessary to deflect the lever and be registered as a lever “contact”. In the

middle of the opposite wall, 1-cm from the top of the chamber, there was a white house light that was illuminated for the duration of each training session.

Rats underwent one day of pre-training in which the food magazine was initially baited with two 45-mg banana-flavored pellets to direct the rats' attention to the site of reward delivery. The house light was turned on after a 5-min acclimation period to the testing chamber, and upon illumination of the house light the pre-training session began and lasted approximately 12.5 minutes. Pre-training sessions consisted of 25 trials, during which the lever remained retracted, but food pellets were randomly delivered into the food magazine, with one pellet delivered per trial on a variable interval 30-second schedule (range 0-60 seconds), for a total of 25 pellets. Following pre-training, rats underwent PCA training sessions with 25 trials per session. Illumination of the house light again signaled session "start". During each trial an illuminated lever (conditioned stimulus, CS) was presented in the chamber for 8 seconds, and immediately upon its retraction a food pellet (unconditioned stimulus, US) was delivered to the adjacent food magazine. These 25 lever-CS/food-US pairings occurred on a variable interval 90-second schedule (range 30-150 seconds), and each session lasted approximately 40 minutes. Rats underwent one training session per day for 5 days, between the hours of 10:00 h and 14:00 h.

Med Associates software recorded the following information: (1) magazine contacts during lever-CS presentation, (2) latency to the first magazine contact during lever-CS presentation, (3) number of lever-CS contacts, (4) latency to the first lever-CS contact during presentation, and (5) the number of magazine contacts between lever-CS presentations (i.e. during the inter-trial interval). These measures allowed for the quantification of the PCA index, which is used to characterize the behavioral phenotype of each rat based on the conditioned response (CR). Information from session 4 and 5 of training were averaged and used to compute

the PCA index as previously described (Meyer et al. 2012). This index incorporates response bias, latency and vigor of each response and ranges from -1 to 1. A score of -1 indicates an extreme goal-tracker (GT) with a CR always directed toward the food magazine upon lever-CS presentation. A score of 1 indicates an extreme sign-tracker (ST) with a CR always directed toward the lever-CS upon presentation. For this study, GTs had scores between -1 to -0.3, STs between 0.3 and 1, and intermediate responders, those that vacillate between contacting the lever or the food magazine during lever-CS presentation, a score between -0.29 to 0.29. Intermediate responders (n=56) were subsequently excluded as this behavioral phenotype was not pertinent to the current goals; but these rats were used for other studies.

Surgical procedures

Following PCA training, all STs and GTs underwent catheterization surgery to place indwelling catheters into the jugular vein for cocaine self-administration, and stereotaxic surgery immediately followed to place cannulas into the anterior and posterior PVT for localized pharmacological inactivation. For catheterization surgery rats were anesthetized using ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and implanted with indwelling jugular vein catheters as previously described (Crombag et al. 2000, Flagel et al. 2003). Ketamine and xylazine were used for this surgery to ensure the rats remained properly anesthetized for the duration of the surgery, and to allow the surgeons to quickly and efficiently implant the catheter. After catheterization surgery rats were given an injection of saline (5 ml, s.c.) to minimize dehydration before undergoing stereotaxic surgery. Once rats were fully ambulatory, they were anesthetized with 5% isoflurane and maintained under anesthesia using 2% isoflurane. Isoflurane was used for this surgery as there was higher risk of the time it takes to complete this surgery going beyond

the time limit that ketamine and xylazine can safely anesthetize a rat. Additionally, the rats recover from isoflurane anesthesia at a faster rate compared to ketamine and xylazine, thus providing a safer means of anesthesia for the second surgery in one day. Rats were fitted into the ear bars of the stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) that was outfitted with a digital manipulator arm (Stoelting, Wood Dale, IL). The scalp was cleaned with ethanol and Betadine solution (Purdue Products, Stamford, CT), and then an incision was made to expose the skull. The skull was then leveled within +/- 0.1 mm of the bregma and lambda coordinates. Chronic guide cannulas (26 gauge, stainless steel; PlasticsOne) were inserted 1 mm above the anterior (relative to bregma: AP -2.0, ML 1.0, DV -4.5) and posterior (relative to bregma: AP -3.0, ML 1.0, DV -4.5) PVT at a 10° angle to the midline to circumvent the superior sagittal sinus and prevent unnecessary bleeding. Due to an initially low success rate of correct injector placement, a subset of rats included in this study had different DV coordinates (relative to bregma: anterior DV -4.6; posterior DV: -4.6), but all other coordinates remained the same. Cannulas were secured to the skull using screws and acrylic dental cement (Ortho-Jet, Lans Dental Manufacturing, Wheeling, IL). A double cannula steel stylet (PlasticsOne) the same length as the guide cannula was inserted into the guide cannula to prevent occlusion. A screw top was put on top of the guide cannula to prevent the rats from removing the stylets.

Rats received an injection of Flunixin (2.5 mg/kg s.c.) and an infusion of gentamicin sulfate (1 mg/ml i.v., 0.2 ml) on the day of surgery and the day following surgery. Rats also received an i.v. infusion of heparin (100 units/ml, 0.05 ml) and gentamicin sulfate (1 mg/ml, 0.05 ml) daily to maintain catheter patency and decrease the chance of infection throughout the cocaine self-administration paradigm. Following surgeries, rats were allowed to recover for a minimum of 10 days, and all sutures and surgical staples were removed during this time. Prior to

the start of the cocaine self-administration paradigm, and before advancing to each subsequent infusion criterion, catheters were checked for patency using methohexital sodium diluted in sterile saline (10 mg/ml i.v., 0.1 ml). If the rat did not exhibit ataxia within 10 seconds of methohexital sodium administration they were removed from the study for loss of catheter patency.

Cocaine self-administration

Cocaine self-administration occurred in the same chambers as PCA training. However, chambers were reconfigured to contain just two nose ports located 4-cm from the grid floor. One nose port was designated “inactive” and one “active”. The active port was on the opposite side of the wall as the lever-CS was during PCA training to minimize side bias. One minute after the program was initiated, the house light was illuminated along with a discrete cue light located in the active port. The discrete cue light in the active port remained on for 20 seconds at the start of each session to direct the rat’s attention to the port. During this time and for the remainder of the session, pokes were recorded in both ports, but only those in the active port resulted in drug infusion (i.e. pokes into the inactive port were without consequence). Reinforcement occurred on a fixed-ratio 1 (FR1) schedule, such that one entry into the active port resulted in a 0.5 mg/kg infusion of cocaine (Mallinckrodt, St. Louis, MO) diluted in 0.9% sterile saline, delivered in 25 µl over 1.6 seconds. Simultaneous with the cocaine infusion, the discrete cue light in the active port was illuminated and stayed on for a total of 20 seconds, during which head entries into the active port are recorded, but without consequence. Infusion criteria (IC) were used to ensure that all rats received the same number of cocaine infusions, and cocaine cue-light pairings (Saunders and Robinson 2010, Saunders and Robinson 2011, Saunders et al. 2013, Flagel et al. 2016). An IC refers to the number of cocaine infusions the rat had to receive to terminate the session

(Saunders and Robinson 2010), and thus the number of cocaine cue-light pairings each rat received (i.e. IC5 means the rat would receive 5 cocaine infusions, and 5 cocaine cue-light pairings, during the session). Once rats met the IC, or after 5 hours, sessions were terminated. Self-administration training occurred once per day between the hours of 8:00 h and 19:00 h for 15 consecutive days using the following schedule: four days at IC5, three days at IC10, three days at IC20 and five days at IC45. In order to move to the next IC rats had to successfully meet each IC for at least 2 consecutive sessions and maintain catheter patency. If these contingencies were not met, the rat was excluded from the study (loss of catheter patency, n=15 (ST: 8, GT: 7); did not meet IC, n=51 (ST: 28, GT: 23)). At IC45, the dose of cocaine was decreased to 0.2 mg/kg/infusion to promote a higher response rate and to encourage rats to reach criterion before the session time limit (Saunders and Robinson 2010). After self-administration training, rats then underwent 14 days of forced abstinence during which they were left undisturbed in the colony room. This time period was chosen as it has been shown to result in an increase in cue-induced drug-seeking behavior compared to shorter periods of abstinence (Grimm et al. 2001).

Extinction training

Extinction training commenced after the 14-day abstinence period. Testing chambers remained in the same configuration as cocaine self-administration, and entries into the active and the inactive port were recorded but without consequence. Thus, head entries into the active port did not result in cocaine delivery nor the presentation of the cue-light. Extinction sessions lasted for 45 minutes and occurred three times a day for six days between the hours of 9:00 h and 17:00 h, for a total of 18 sessions. The last three extinction sessions occurred the same day as the test for cue-induced reinstatement. In order to undergo the test for cue-induced reinstatement rats

must have completed the 18 extinction sessions and have fewer than 10 entries into the active port during each of the last two sessions, which all rats included in final analysis accomplished. Before the last extinction training session (session 18) the cannula "dust" cap and stylet were removed, the injector was inserted into the cannula and removed, and then the stylet and cap were put back into place to habituate the rats to the injection procedure that would occur prior to the test for cue-induced reinstatement.

Cue-induced reinstatement test

The cue-induced reinstatement test occurred immediately following the last extinction training session (e.g. session 18). Rats were counterbalanced into two different drug treatment groups based first on PCA score. Within each group, rats were further counterbalanced based on the number of port entries during self-administration sessions and behavior during the extinction sessions. Treatment groups received either a mixed cocktail of agonists to the GABA-B (baclofen) and GABA-A receptors (muscimol; Sigma-Aldrich, St. Louis, MO), or a saline injection (control group). Baclofen/ muscimol (B/M) was given at a dose of 6 pmol/nl and 0.6 pmol/nl respectively, as infusion of this dose into the PVT has previously been shown to affect cocaine-seeking behavior (Browning et al. 2014, Matzeu et al. 2015). Injections occurred in a room adjacent to the testing room and were administered using a standard dual infusion pump (Pump 11 Elite, Harvard Apparatus) with P50 tubing connecting the two 1-µl syringes (Hamilton) to the injector (33 gauge with a 1-mm projection; Plastics One). Injections occurred at a rate of 100 nl/min for two minutes (total of 200 nl volume), and the injector was left in place for an additional two minutes to allow the drug to diffuse away from the injector and throughout the PVT (Browning et al. 2014). Following the injection, the stylet and cap were replaced, and

the rat was brought into the testing room and placed into the Med Associates testing chamber. The house light came on one minute after program initiation, and head entries into the active and inactive port were recorded for the duration of the session. During the cue-induced reinstatement test, head entries into the active port resulted in the presentation of the cue-light for 20 seconds (same as in self-administration training), but no cocaine infusion. That is, presentation of the cue-light previously associated with drug delivery acted as a conditioned reinforcer, and entries into the active port were used as a measure of cocaine-seeking behavior. Entries into the inactive port were recorded, but without consequence. Sessions terminated after 45 minutes, and testing occurred between the hours of 15:00 h and 17:00 h.

Locomotor testing

A subset of rats (9 STs, 13 GTs) were assessed for the effects of PVT inactivation on general locomotor activity. The day after the cue-induced reinstatement test rats were put into a locomotor testing chamber (43 x 21.5 cm floor area, 25.5 cm high) outfitted with infrared beams mounted 2.3 and 6.5 cm above the grid floor to track lateral and rearing movements, respectively. All testing occurred under red light between the hours of 12:00 and 16:00. Rats underwent a 45-minute habituation period for which they were placed into the locomotor testing chamber and left undisturbed, but activity was recorded. Following the conclusion of habituation, rats were removed from the test chamber and given the same drug infusions (i.e. B/M or saline) they received prior to the reinstatement test on the preceding day. All infusion procedures were identical to those for the cue-induced reinstatement test, with injections occurring at a rate of 100 nl/min for 2 minutes (total of 200 nl volume) and the injector left in place for an additional 2 minutes (Browning et al. 2014). Rats were then placed back into the

locomotor testing chamber and underwent a 45-minute test session. For both the habituation and test session, lateral and rearing locomotor movements were recorded in 5-minute increments and cumulative locomotor activity was calculated based on the sum of these movements across the 45-min session. Once the session was complete for all of the rats, rats were removed from the test chambers and placed back into their home cages in the colony room.

Histology

After all testing was complete, rats were anesthetized with ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and subsequently received an infusion of 2% Chicago Sky Blue dye (200 nl total at a rate of 100 nl/min; Sigma-Aldrich, St. Louis, MO) into the PVT in order to identify the injection site. Rats then underwent transcardial perfusion with 0.9% saline followed by 4% formaldehyde at 4°C (pH= 7.4) with an injector still inserted into the guide cannula. Brains were extracted and remained in formaldehyde for 24 hours at 4°C. Brains were then cryoprotected for 24 hours in graduated sucrose solutions (10%, 20% then 30% sucrose in phosphate buffer, pH= 7.4) at 4°C over the course of 3 days. Brains were encased in Tissue-Plus O.C.T. (Fisher HealthCare, Houston, TX), frozen using dry ice and sectioned coronally on a cryostat at a thickness of 40 µm. After sectioning, brains were mounted and stained using Eosin-Y (Sigma-Aldrich, St. Louis, MO), dehydrated with ethanol solutions, exposed to three xylene washes and then coverslipped with Permount (Fisher Scientific, Fair Lawns, NJ). Verification of injection sites was done using a Leica DM1000 light microscope (Buffalo Grove, IL). Two experimenters, blind to group assignments, scored the injector sites as being within or outside of the boundaries of the PVT for both the anterior (relative to bregma: AP: -1.8 to -2.28) and posterior (relative to bregma: AP: -2.76 to -3.24) PVT sites with the guidance of a rat brain

stereotaxic atlas (Paxinos G 2007). Only rats in which both scorers agreed on having correct injector placement within the PVT boundaries were included in the final analyses as indicated below.

Statistical analysis

All PCA training, cocaine self-administration and extinction training sessions were analyzed using a linear mixed-effects model with SPSS Statistics Program (Statistical Package for the Social Sciences), version 22 (IBM, Armok, NY, USA). The best covariance structure was selected using the lowest Akaike's information criterion for each dataset. Behavior during the cue-induced reinstatement test was analyzed using a three-way ANOVA. To compare behavior during the last extinction session to that during the cue-induced reinstatement test, a repeated-measures ANOVA was used. A repeated measures ANOVA was also used to analyze differences in locomotor activity between the habituation and test session for the locomotor activity test. All ANOVAs were performed using StatView, version 5.0 (SAS Institute Inc., Cary, NC, USA). To determine if there was a significant relationship between the rate of extinction and cue-induced reinstatement, a quadratic regression model was fit to each rat's extinction training curve. The intercept, linear and quadratic term were then regressed onto the number of pokes into the active port during the reinstatement test. Importantly, this analysis accounts for differences in extinction behavior that may otherwise confound behavior during the reinstatement test. These analyses were carried out using SPSS, version 22. Statistical significance was set at $p<0.05$ for all tests. When significant main effects or interactions were detected post-hoc analyses were conducted using Bonferroni tests to correct for multiple comparisons.

Results

Histology

Fig. 2.2 shows a map of the cannula placements of the rats with accurate placements in the anterior and posterior PVT. Of those rats that successfully completed the behavioral portion of the study, only those with correct cannula placement in both the anterior and posterior PVT were included in final analysis (ST Saline, n=10; ST B/M, n=11; GT Saline, n=11; GT B/M, n=10).

Pavlovian conditioned approach (PCA) training

PCA behavior was analyzed across training sessions using the following dependent variables: probability to contact the lever or magazine, the number of lever or magazine contacts, and latency to contact the lever or magazine. Phenotype (ST or GT), Treatment (B/M or saline) and Session were used as the independent variables. For all measures (see Fig. 2.3) there was a significant Effect of Phenotype, Effect of Session and a Phenotype x Session interaction ($p<0.05$). Relative to GTs, rats characterized as STs showed a greater probability to contact the lever ($F_{1,43} = 172.19$, $p<0.001$), a greater number of contacts with the lever ($F_{1,38} = 122.78$, $p<0.001$), and a lower latency to contact the lever ($F_{1,42} = 136.61$, $p<0.001$) (Fig. 2.3a-c). Post-hoc analyses revealed a significant difference between phenotypes on all five sessions for these measures ($p<0.001$). In contrast, GTs showed a greater probability to contact the food magazine ($F_{1,42} = 48.02$, $p<0.001$), a greater number of contacts with the food magazine ($F_{1,41} = 56.97$, $p<0.001$), and a lower latency to contact the food magazine ($F_{1,38} = 46.20$, $p<0.001$) compared to STs (Fig. 2.3d-f). Post-hoc analyses revealed a significant difference between phenotypes for the probability to contact the food magazine and the number of magazine contacts during sessions

two through five ($p<0.05$), and differences in the latency to contact the food magazine during sessions three through five ($p<0.001$). There were no significant differences between Treatment groups, nor were there significant interactions with this variable, even when phenotypes were analyzed separately. This is to be expected as groups were balanced based on their PCA behavior, and treatment did not occur during this phase of the experimental design (see Fig. 2.1).

STs and GTs do not differ in the acquisition of cocaine self-administration

Cocaine self-administration behavior was analyzed across IC using nose pokes as the dependent variable, and Phenotype (ST or GT), Treatment (B/M or saline), and Port (active or inactive) as the independent variables. As shown in Fig. 2.4a, all rats discriminated between the active and inactive port (Effect of Port, $F_{1,76} = 175.62$, $p<0.001$) and increased their responding into the ports at each successive IC (Effect of IC, $F_{3,76} = 60.48$, $p<0.001$). There was also a significant IC x Port interaction ($F_{3,76} = 61.12$, $p<0.001$), indicating that responses into the active port increased across IC ($F_{3,76}=121.07$, $p<0.001$), while responses into the inactive ports did not change across IC, as to be expected. Indeed, rats successfully differentiated between the two ports at every stage of training (Effect of Port, IC5: $p=0.002$; IC10: $p<0.001$; IC20: $p<0.001$; IC45: $p<0.001$). There were no significant Effects of Treatment ($F_{1,76} = 1.90$, $p=0.17$) nor Phenotype ($F_{1,76} = 1.46$, $p=0.23$), and no significant interactions with these variables. These data are consistent with those reported in previous studies showing that STs and GTs do not differ from one another in the acquisition of cocaine self-administration using these doses of cocaine and the IC paradigm (Saunders and Robinson 2010, see also Beckmann et al. 2011).

STs and GTs do not differ in the rate of extinction.

Extinction behavior was analyzed across training days using nose pokes (average of the 3 sessions per day) as the dependent variable, and Phenotype (ST or GT), Treatment (B/M or saline), and Port (active or inactive) as the independent variables. Cocaine-seeking behavior decreased with repeated extinction training days (Effect of Day, $F_{5,76} = 23.85$, $p<0.001$) (Fig. 2.4b). A significant Effect of Port ($F_{1,86} = 55.63$, $p<0.001$) showed that rats differentiated between the active and inactive port (Fig. 2.4b). However, a significant Day x Port interaction ($F_{5,76} = 7.44$, $p<0.001$) revealed that as extinction training progressed rats stopped preferring the active port over the inactive port; this was especially evident later in training as nose pokes into the active port decreased (Fig. 2.4b). There was not a significant Effect of Treatment ($F_{1,86} = 1.26$, $p=0.27$), nor was there a significant Effect of Phenotype ($F_{1,86} = 1.11$, $p=0.30$). There was, however, a significant interaction between Phenotype and Day ($F_{5,76}= 2.88$, $p=0.02$). Post-hoc analyses revealed that STs and GTs differ from one another in extinction behavior during the second ($p=0.03$) and fourth ($p=0.01$) training days. Yet, when each extinction session was included in the analysis (rather than averaging across the three sessions per day) there was not a significant Effect of Phenotype ($F_{1,86} = 1.49$, $p=0.23$), nor any significant interactions with this variable. These findings are in agreement with those reporting that STs and GTs do not differ in their rate of extinction of instrumental drug-taking behavior (Saunders and Robinson 2011, Ahrens et al. 2016).

Inactivation of the PVT affected cue-induced cocaine-seeking behavior selectively in GTs

Drug-seeking behavior during the cue-induced reinstatement test was analyzed using nose pokes as the dependent variable, and Phenotype (ST or GT), Treatment (B/M or saline), and Port (active or inactive) as the independent variables. Rats differentiated between the active and

inactive port during cue-induced reinstatement (Effect of Port, $F_{1, 76} = 51.48$, $p<0.001$), with all groups showing a preference for the active port compared to the inactive port (active vs. inactive for each group, $p<0.03$) (Fig. 2.5a). There was an overall Effect of Treatment ($F_{1,76} = 4.53$, $p=0.04$), and a significant Phenotype x Treatment interaction ($F_{1,76} = 5.09$, $p=0.03$), suggesting that PVT inactivation differentially affected the responding of STs and GTs at both ports. In GTs, PVT inactivation resulted in a greater number of nose pokes into the active ($p=0.02$) and inactive port ($p=0.04$) compared to GT controls. Inactivation of the PVT in STs had no effect on responses in either port compared to ST controls; but responding in the active port was significantly different between STs and GTs following PVT inactivation ($p<0.05$; Fig. 2.5a). This latter effect is due to the significant increase in drug-seeking behavior in GTs following B/M. It should be noted, however, that, in contrast to previous studies (Saunders and Robinson 2010, Saunders et al. 2013), the ST control group did not show significantly greater cocaine seeking compared to GT controls ($p=0.38$). Nonetheless, these data highlight a role for the PVT in mediating cue-induced drug-seeking behavior in GTs.

To account for the differences in responding in the inactive port in GTs that received B/M relative to those that received saline, we subtracted the number of responses in the inactive port from those in the active port as an index of drug-seeking behavior during the last extinction session and during the cue-induced reinstatement test. This index was then analyzed across sessions (i.e. extinction vs. reinstatement) with Phenotype (ST or GT) and Treatment (B/M or saline) as the independent variables. This analysis revealed that all groups showed enhanced cocaine-seeking behavior during the reinstatement test relative to behavior during the last extinction training session (Effect of Session, $F_{1,38} = 51.42$, $p<0.0001$) (Fig. 2.5b). A significant Phenotype x Treatment interaction ($F_{1,38} = 5.12$, $p=0.03$) after “correcting” for differences in

pokes into the inactive port, indicates enhanced cue-induced cocaine-seeking behavior in GTs following PVT inactivation (p=0.03; Fig. 2.5b). These findings are also illustrated in Fig. 2.5c and 2.5d, which show individual differences in responding during extinction and reinstatement for GTs treated with saline (Fig. 2.5c) relative to those treated with B/M (Fig. 2.5d). Taken together, these data demonstrate a key role for the PVT in mediating the propensity for cue-induced drug-seeking behavior in this phenotype.

Rate of extinction predicts cue-induced reinstatement of cocaine-seeking behavior in control groups

We found that the rate of decrease in responses in the active port during extinction training (i.e. extinction rate) predicted the number of responses into the active port during cue-induced reinstatement (Fig. 2.6). Specifically, for STs, a faster decrease in pokes into the active port during extinction training resulted in a lower number of pokes into the active port during reinstatement ($F_{1,8}$ = 9.215, p=0.02; quadratic term= -129.94; Fig. 2.6a). In contrast, for GTs, a faster extinction rate resulted in a greater number of pokes into the active port during reinstatement ($F_{1,8}$ = 9.176, p=0.01; quadratic term= 43.79; Fig. 2.6b). Importantly, the significant relationship between the rate of extinction and cue-induced drug-seeking behavior was only present in the control groups. That is, PVT inactivation obscured the significant relationship between these variables for both STs ($F_{1,8}$ = 0.78, p=0.40; Fig. 2.6c) and GTs ($F_{1,8}$ = 1.52, p=0.25; Fig. 2.6d). These data further highlight the notion that GTs and STs capture different forms of reward learning, both of which may be relevant to addiction liability (Saunders and Robinson 2010, Saunders et al. 2013, Saunders et al. 2014, Kawa et al. 2016, Pitchers et al.

2017), and both of which appear to be mediated by the PVT (Haight and Flagel 2014, Haight et al. 2015, Haight et al. 2017).

Inactivation of the PVT does not affect general locomotor activity

To assess whether PVT inactivation had any effects on general locomotor activity, rats were first allowed to habituate to the locomotor testing chamber and then received either saline or B/M (same treatment as that prior to the reinstatement test) before being placed back into the chamber. Locomotor activity was analyzed across sessions (habituation or test) with Phenotype (ST or GT) and Treatment (B/M or saline) as the independent variables. There was not a significant effect of Phenotype ($F_{1,18}$=0.63, p=0.44), nor a significant effect of Treatment ($F_{1,18}$=0.028, p=0.87). There was, however, a significant effect of Session (Effect of Session, $F_{1,18}$ = 35.15, p<0.0001; Fig. 2.7). As evident in Fig. 2.7, there was an overall decrease in locomotor activity during the test session relative to the habituation session. This is likely due to an attenuation in novelty-induced locomotion after habituation to the testing chamber. There was also a significant Session x Treatment interaction ($F_{1,18}$ = 8.38, p=0.01) suggesting that the effects of treatment differed between habituation and test sessions, but not between phenotypes. Post-hoc comparisons did not reveal any additional significant effects. Thus, transient inactivation of PVT does not appear to affect general locomotor activity.

Discussion

In the current study, we assessed the role of the PVT in cue-induced reinstatement of cocaine-seeking behavior using an animal model that captures individual variation in the propensity to attribute incentive salience to reward-cues. It is well-established (Robinson and

Flagel 2009, Robinson et al. 2014) that both goal-tracker and sign-tracker rats attribute predictive value to reward-cues, but sign-trackers also attribute enhanced incentive motivational value to these cues, which relies on different neural mechanisms (Flagel et al. 2011a, Flagel et al. 2011b, Yager et al. 2015, Haight et al. 2017). The PVT has been identified as a central node that may mediate both predictive and incentive learning via its multiple interconnected neural networks (Flagel et al. 2011a, Haight et al. 2017). In addition, this nucleus has been implicated in response to drugs of abuse and in the reinstatement of drug-seeking behavior (Deutch et al. 1995, Deutch et al. 1998, Stephenson et al. 1999, Hamlin et al. 2009, James et al. 2010, James et al. 2011, Browning et al. 2014, Yeoh et al. 2014, Matzeu et al. 2015, Matzeu et al. 2016, Matzeu et al. 2017). The role of the PVT in encoding the motivational value of a cue light previously associated with cocaine delivery was assessed here in STs and GTs. During the cue-induced reinstatement test, responses into the port that previously resulted in drug delivery, now resulted in presentation of the drug-cue-light. Inactivation of the PVT resulted in a robust increase in cocaine-seeking behavior during this test, but selectively in GTs compared to controls of the same phenotype. Importantly, this effect held true in GTs even after accounting for differences in responding in the inactive port following PVT inactivation, and these differences do not appear to be due to gross changes in locomotor activity. Although PVT inactivation did not significantly affect cue-induced drug-seeking behavior in STs compared to controls of the same phenotype, this manipulation did result in a difference between the phenotypes. That is, following PVT inactivation, STs show attenuated responding relative to GT, but this effect is primarily due to the significant increase in responding following PVT inactivation in GTs. These and other findings (Haight et al. 2015, Haight et al. 2017) suggest that, for GTs, the PVT may act as a

"brake" on the incentive motivational properties of reward cues and removal of this "brake" unmasks the incentive value of such cues, thereby evoking maladaptive cue-driven behaviors.

The design of this experiment was such that it minimized the likelihood of any prior behavioral testing affecting the outcomes of PVT inactivation on cue-induced reinstatement. STs and GTs did not differ from one another in cocaine self-administration behavior. These data are consistent with previous results using this schedule of training (Saunders and Robinson 2010, Saunders and Robinson 2011, Saunders et al. 2013, Flagel et al. 2016), which ensured that all rats received the same number of drug-cue pairings during self-administration. In addition, there were no significant differences between phenotypes in the rate of extinction of drug-seeking behavior (when session was considered as the repeated variable), which is also consistent with previous studies (Ahrens et al. 2016). However, an additional analysis revealed that the rate of extinction did affect responding during the cue-induced reinstatement test, and did so differentially for GTs and STs. For GTs, the faster the rats decreased responding into the active port during extinction, the greater the number of pokes into the active port during the reinstatement test. In contrast, for STs, a faster decrease in responding during extinction resulted in fewer pokes into the active port during reinstatement. This differential relationship between the rate of extinction and subsequent cue-induced drug-seeking behavior in GTs and STs has not been previously reported, but further highlights the distinct learning mechanisms that may underlie different forms of addiction liability in these two phenotypes (Saunders and Robinson 2010, Saunders et al. 2013, Saunders et al. 2014, Kawa et al. 2016, Pitchers et al. 2017). Moreover, the fact that these relationships were obscured in both phenotypes following inactivation of the PVT suggests that this nucleus is important for linking prior experiences with subsequent behavior, and does so via its differential role in the learning mechanisms underlying

individual variation in cue-motivated behaviors (Haight and Flagel 2014, Haight et al. 2015, Haight et al. 2017).

Prior studies have reported that STs show greater cue-induced reinstatement of cocaine-seeking behavior compared to GTs (Saunders and Robinson 2010, Saunders et al. 2013), but this finding was not replicated in the current study, perhaps due to methodological differences. Here we enforced a two-week abstinence period during which the rats remained undisturbed, whereas prior studies using the ST/GT model used a one-month abstinence period. Although the two-week period has been shown to result in robust drug-seeking behavior compared to shorter time periods (Grimm et al. 2002), the one-month abstinence period is known to even further enhance cue-induced drug-seeking behavior (Grimm et al. 2001). Indeed, it appears that longer abstinence periods that permit robust "incubation of craving" effects (Grimm et al. 2001) are required to reveal enhanced cue-induced drug-seeking behavior in STs relative to GTs (Saunders and Robinson 2010). Although we did not observe ST/GT differences in reinstatement behavior in the current study, we did find that a two-week abstinence period was sufficient to elicit cue-induced drug-seeking behavior, and, importantly, to capture the effects of PVT inactivation on individual differences in this behavior. It should also be noted that those studies previously reporting differences in cue-induced drug-seeking behavior between STs and GTs implemented extinction training prior to the abstinence period (Saunders and Robinson 2010, Saunders et al. 2013). Conversely, in the current study, extinction occurred after abstinence, and immediately preceding the test for reinstatement. This is especially noteworthy given the differential relationship revealed between the rate of extinction and cue-induced drug-seeking behavior in control STs and GTs in the current study. It will be important for future studies to further investigate this relationship and to systematically examine individual variation in cue-induced

drug-seeking behavior following various extinction training procedures and forced abstinence periods.

Decreasing neuronal transmission in the PVT has previously been shown to result in a robust decrease in drug-seeking behavior following cue- (Matzeu et al. 2015), drug- (James et al. 2010) or context-induced (Hamlin et al. 2009) reinstatement. In contrast, here we report an increase in cue-induced drug-seeking behavior following PVT inactivation, but selectively in GTs. These seemingly discrepant findings are likely due to a combination of factors, including the type of reinstatement models that were used and the incorporation of individual differences in the current experimental design. Indeed, it is well-established that different forms of reinstatement recruit different neural circuits (Shaham et al. 2003, Kalivas and Volkow 2005, Crombag et al. 2008, Khoo et al. 2017) and that drug-associated stimuli engage brain regions, including the PVT, to a different degree in STs and GTs (Yager et al., 2015). Furthermore, relative to STs, GTs are more prone to reinstatement elicited by contextual cues (Saunders et al. 2014), and forebrain cholinergic activity appears to mediate this vulnerability (Pitchers et al. 2017). Thus, it is conceivable that the PVT plays a role in both cue- and context-induced reinstatement (Hamlin et al. 2009, Matzeu et al. 2015), but the form of the reinstatement "trigger" and inherent differences in the propensity to attribute incentive motivational value to said "triggers" determine its exact role, which is dependent upon the circuitry involved. We postulate that projections from the prelimbic cortex to the PVT are particularly important in mediating the attribution of incentive salience to reward cues (Flagel et al. 2011a, Paolone et al. 2013, Haight et al. 2017, Pitchers et al. 2017), and ongoing studies are investigating the role of this circuit in cue- vs. context-induced reinstatement in STs and GTs.

Another methodological detail that likely contributed to the present findings is the fact that both the anterior and posterior regions of the PVT were simultaneously inactivated in the current study; whereas some of the prior studies examining the role of the PVT in drug-seeking behavior targeted just one of these sub-regions (Hamlin et al. 2009, Matzeu et al. 2015, Matzeu et al. 2016). Importantly, these two sub-regions are known to differ in their afferent and efferent connections (Li et al. 2008, Li and Kirouac 2012, Hsu et al. 2014, Kirouac 2015, Dong et al. 2017). While both sub-regions project to the NAc, the anterior PVT (aPVT) sends a denser projection to the NAc shell (Dong et al. 2017). When Hamlin and colleagues (Hamlin et al. 2009) demonstrated a role for the PVT in context-induced reinstatement, they also showed that this renewal of drug-seeking behavior engaged the PVT-NAc shell pathway, which included the entire rostra-caudal extent of the PVT. Additionally, the PVT-NAc pathway is involved in the acquisition of cocaine self-administration (Neumann et al. 2016), as well as mediating symptoms during drug withdrawal (Zhu et al. 2016). Recently, however, it was shown that pharmacological inactivation of the anterior, but not the posterior, PVT increases sucrose-seeking behavior upon reward omission, and that this behavior is specifically mediated by aPVT projections to the NAc shell (Do-Monte et al. 2017). In contrast, differences in food-cue-induced neuronal activity between STs and GTs seems to be restricted to cells projecting from the posterior PVT (pPVT) to the “shore” (area bordering the core/shell) of the nucleus accumbens (Haight et al., 2017). The pPVT receives dense orexinergic projections from the lateral hypothalamus (LH) (Kirouac et al. 2005), and antagonism of orexin 2 receptors in this subregion decreases drug-seeking behavior (Matzeu et al., 2016). Relative to GTs, STs show enhanced food-cue-induced neuronal activity in cells projecting from the LH to the PVT (Haight et al. 2017), and orexin receptor antagonism in the PVT appears to decrease the incentive value of reward cues (Haight 2016). Taken together,

these data support the notion that distinct neuronal networks within the PVT , presumably related to rostra-caudal subdivisions and corresponding circuitry, differentially mediate appetitive and addiction-related behaviors (for review and further discussion see Millan et al. 2017). Thus, it is conceivable that selective inactivation of either the aPVT or pPVT would have different effects on cue-induced drug-seeking behavior in STs and GTs than those in the current study, for which the entire PVT was targeted. Based on the findings described above, we hypothesize that selective inactivation of the posterior PVT would attenuate cue-induced drug-seeking behavior in STs relative to controls of the same phenotype, an effect that was not observed here. Given the complex and heterogeneous circuitry of the PVT, ongoing studies are exploiting chemogenetic tools to better elucidate the role of specific circuits and cell types within this nucleus in drug-seeking behavior.

The sign-tracker/goal-tracker animal model has allowed us to parse the incentive from the predictive value of reward cues and to begin to identify the neural networks underlying these distinct forms of learning (Flagel et al. 2011a, Yager et al. 2015, Flagel and Robinson 2017, Haight et al. 2017). Most studies to-date that have exploited this model of individual variation to study the underlying brain mechanisms have focused on neuronal responses to food-cues that were attributed with incentive or predictive value following classical Pavlovian conditioning paradigms (Flagel et al. 2010, Flagel et al. 2011b, Haight and Flagel 2014, Haight et al. 2017). In the current study, however, we targeted a specific nucleus that had been identified as a key player in these Pavlovian learning processes (Flagel et al. 2011a, Haight and Flagel 2014, Haight et al. 2015, Yager et al. 2015, Haight et al. 2017), to determine whether the same nucleus acts to encode the incentive value of a cue that was previously paired with operant drug delivery. While it is known that the neural circuitry mediating Pavlovian conditioning can differ from that

mediating instrumental behavior (Ostlund et al. 2007, Yin et al. 2008, Wassum et al. 2011), the paraventricular nucleus of the thalamus appears to be involved in both (Hamlin et al. 2009, James et al. 2010, Browning et al. 2014, Haight et al. 2015, Matzeu et al. 2015, Neumann et al. 2016, Do-Monte et al. 2017, Matzeu et al. 2017, Otis et al. 2017). The current findings support a role for this nucleus in the attribution of incentive value to reward cues and suggest that, in a subset of individuals, the PVT acts to suppress the learned incentive value of such cues. That is, there is likely a mechanism in place for all individuals to attribute incentive motivational value to reward cues, but only for some individuals is this incentive value revealed. In our model, the PVT appears to "mask" the incentive value for GTs and encode the incentive value for STs (Haight et al. 2015, Haight et al. 2017). The exact mechanism by which this occurs is not yet known, but prior and ongoing studies in our lab suggest that projections from the prelimbic cortex to the PVT may act to inhibit the incentive value of reward cues in GTs, likely via downstream effects on PVT-NAc shell projections. In contrast, in STs, the subcortical hypothalamic-PVT-striatal pathway presumably overrides any "top-down" cortical inhibition, allowing for the encoding of incentive value during learning and subsequent expression of resultant behaviors.

In conclusion, the results of the current study further support the notion that the PVT acts as a central node that differentially regulates cue-motivated behaviors in STs and GTs. These findings extend prior work, demonstrating a role for this nucleus in mediating the incentive value of drug-paired cues following an instrumental paradigm. Inactivation of the PVT enhances cue-induced drug-seeking behavior, but only in GTs relative to controls of the same phenotype. Thus, in GTs, the PVT appears to inhibit the expression of the incentive motivational value of a cocaine-associated cue-light, resulting in suppression of drug-seeking behavior during cue-

induced reinstatement. The fact that inactivation of the PVT results in a difference in cue-induced drug-seeking behavior between STs and GTs, suggests that this nucleus also plays a role in encoding the incentive value of drug-cues for STs, albeit to a different degree and likely via a different neural circuit. Future studies are warranted to better elucidate the neural circuits underlying individual variation in cue-motivated and addiction-related behaviors, and the role of the PVT within these circuits.

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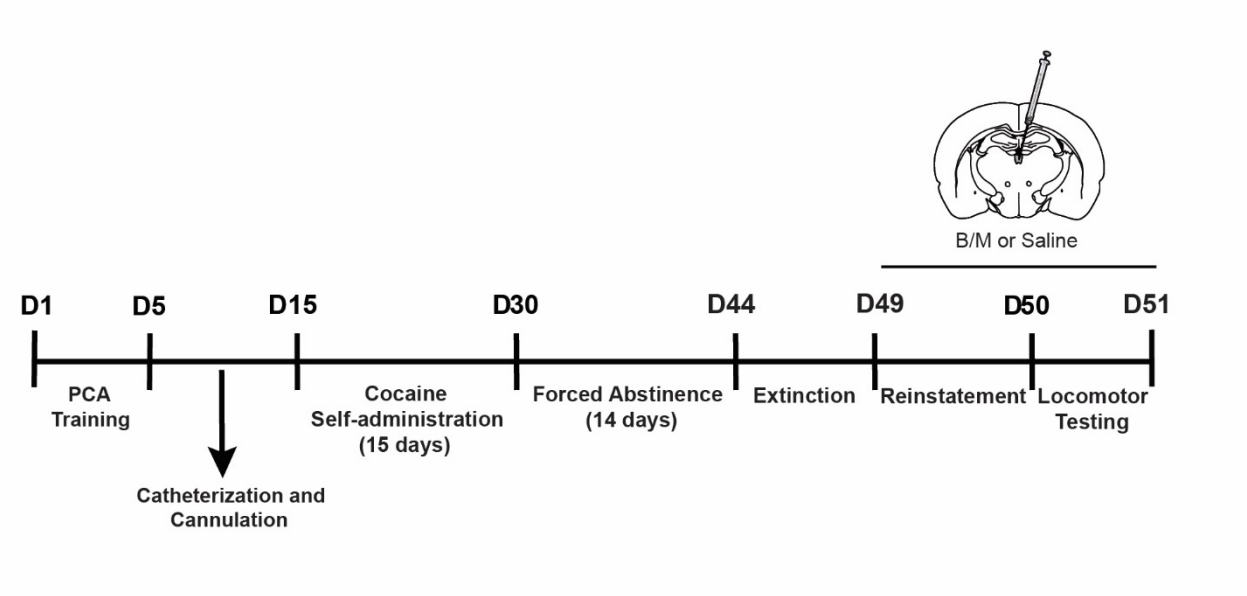

Fig. **2.1** Experimental timeline. Rats underwent Pavlovian conditioned approach (PCA) training and were then implanted with indwelling jugular catheters and double cannula into the anterior and posterior PVT. Cocaine self-administration (15 days), forced abstinence (14 days) and extinction training (18 sessions) followed. Rats were given an injection of either of baclofen/ muscimol (B/M, 6/0.6 pmol/nl) or saline into the PVT prior to cue-induced reinstatement and the locomotor test. The total duration of the study was approximately 50 days.

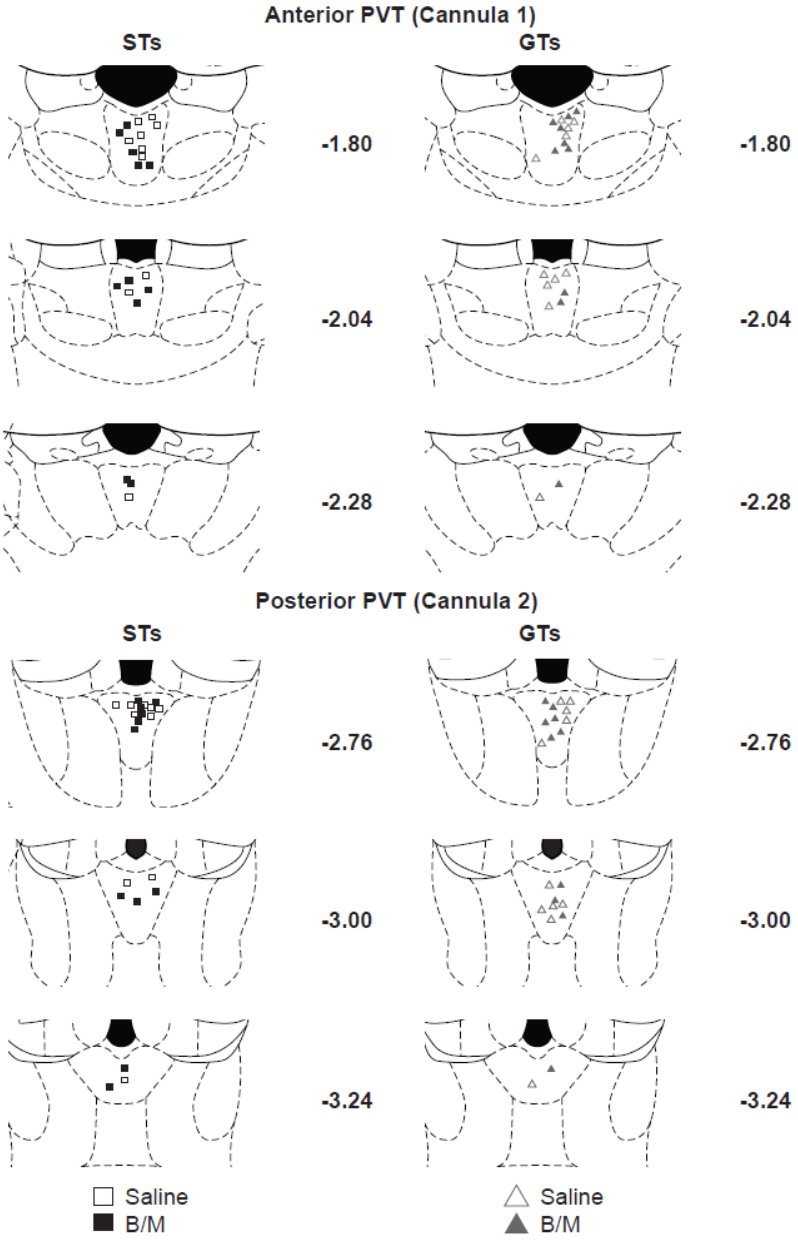

Fig. 2.2 Representation of double cannula placements in the anterior (cannula 1) and posterior (cannula 2) portions of the PVT with respect to bregma. Only those rats considered to have successful cannula placements are included and shown separated by phenotype (STs, left; GTs, right) and treatment group (open symbols saline; closed symbols baclofen/muscimol). (ST Saline, n=10; ST B/M, n=11; GT Saline, n=11; GT B/M, n=10)

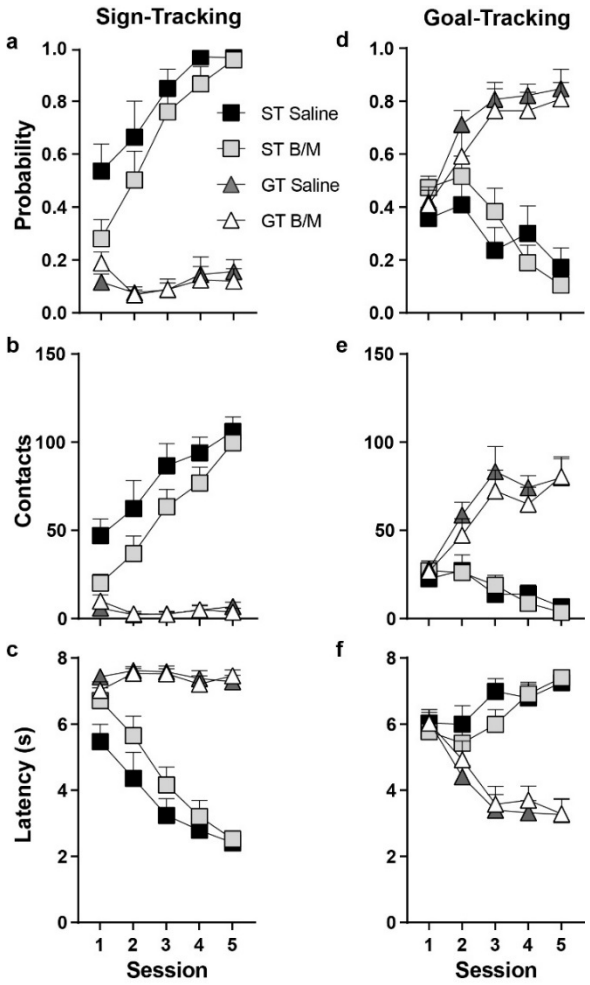

Fig. 2.3 Individual variation in the acquisition of Pavlovian conditioned approach training. Mean + SEM for (a) probability to contact the lever, (b) lever contacts, (c) latency to contact the lever, (d) probability to contact the food magazine, (e) food magazine contacts, and (f) latency to contact the food magazine across 5 Pavlovian conditioning sessions. Rats with a conditioned response directed toward the lever were classified as STs (Saline, n=10; B/M, n=11), and rats with a conditioned response directed toward the food magazine were classified as GTs (Saline, n=11; B/M, n=10). Rats are separated by their test day treatment (saline or B/M (baclofen/muscimol)), but did not receive treatment prior to or during Pavlovian conditioning.

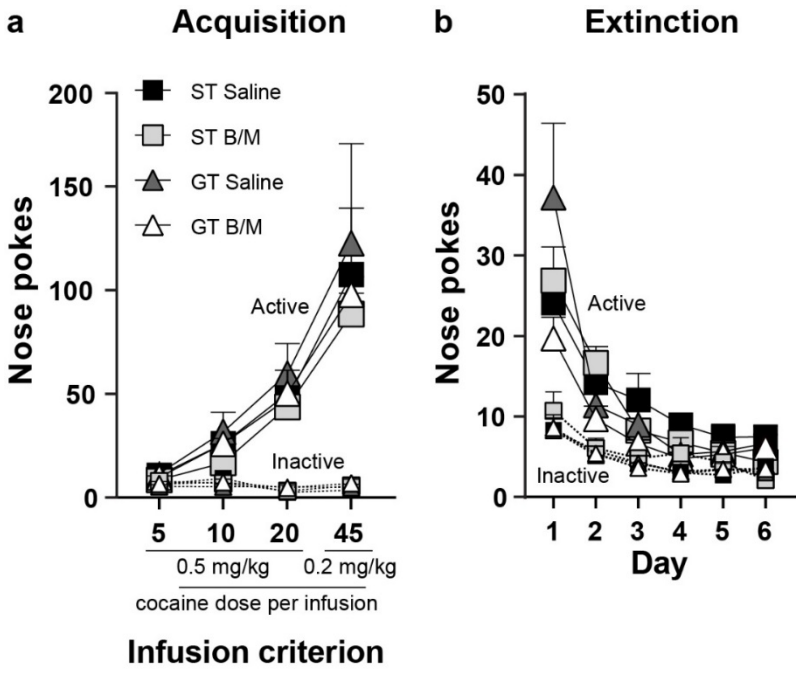

Fig. 2.4 Acquisition and extinction of cocaine self-administration. (a) Mean + SEM for nose pokes into the active and inactive ports across four infusion criterion (IC) in STs (Saline, n=10; B/M, n=11) and GTs (Saline, n=11; B/M, n=10). All rats differentiated between the active and inactive port ($p<0.0001$) across each IC ($p<0.0001$), and there were no significant differences between phenotype or treatment groups (saline or B/M). The cocaine dose at IC5, IC10 and IC20 was 0.5 mg/kg/infusion, and at IC45 it was 0.2 mg/kg/infusion. (b) Mean + SEM for nose pokes into the active and inactive ports for STs (Saline, n=10; B/M, n=11) and GTs (Saline, n=11; B/M, n=10) across extinction training days (3 sessions per day). Rats decreased cocaine-seeking behavior throughout extinction training ($p<0.0001$), regardless of phenotype or assigned treatment group for the subsequent reinstatement test (saline or B/M). A Day x Phenotype interaction ($p=0.20$) was present, however when behavior is analyzed per session, and not grouping sessions into a day, this relationship is no longer present.

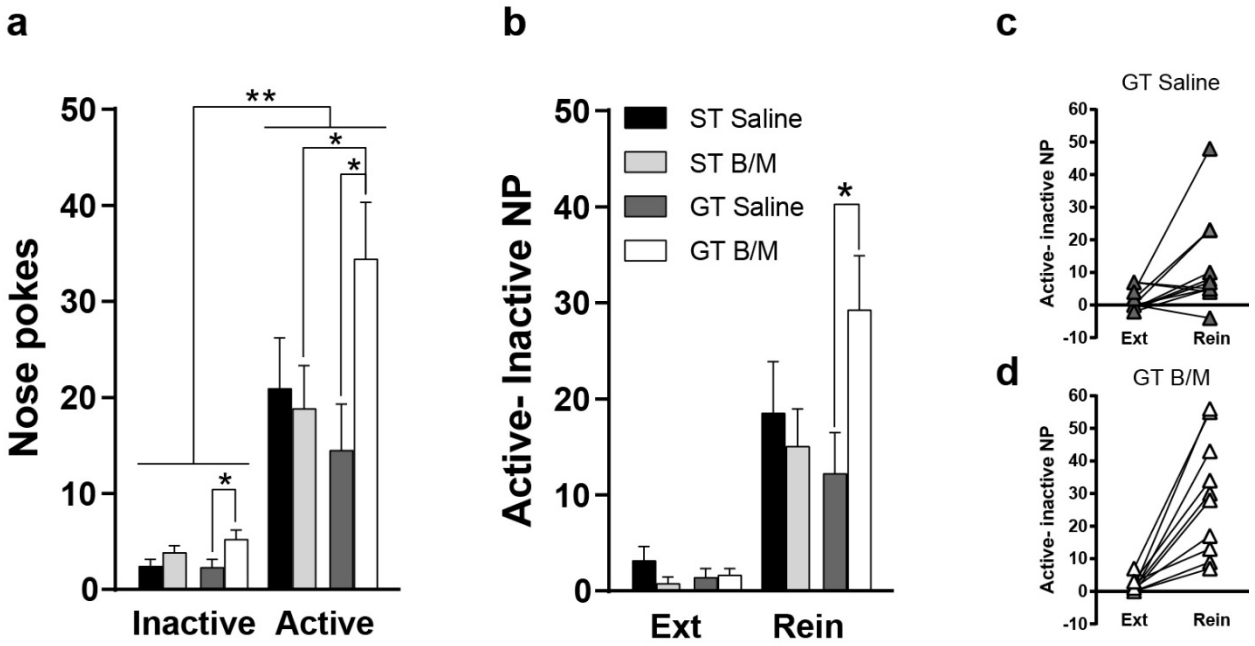

Fig. 2.5 Effects of transient inactivation of the PVT on cue-induced reinstatement of drug-seeking behavior. (a) Mean + SEM of nose pokes into the active and inactive port during cue-induced reinstatement. There was a significant Effect of Port ($p<0.001$), Effect of Treatment ($p=0.04$) and a significant Phenotype x Treatment interaction ($p=0.03$). PVT inactivation resulted in greater drug-seeking behavior in GTs compared to GT controls ($p=0.02$), and a significant difference in drug-seeking behavior between STs and GTs ($p<0.05$). (b) Mean + SEM active-inactive nose pokes (NP) during the last extinction session (Ext) and cue-induced reinstatement (Rein). PVT inactivation resulted in greater drug-seeking behavior in GTs compared to GT controls when accounting for an increase in pokes into the inactive port ($p=0.03$). Mean + SEM active-inactive NP during the last extinction session (Ext) and cue-induced reinstatement (Rein) for (c) each GT rat in the saline group and (d) each GT rat in the B/M group. (ST Saline, n=10; ST B/M, n=11; GT Saline, n=11; GT B/M, n=10) *$p<0.05$, **$p<0.01$

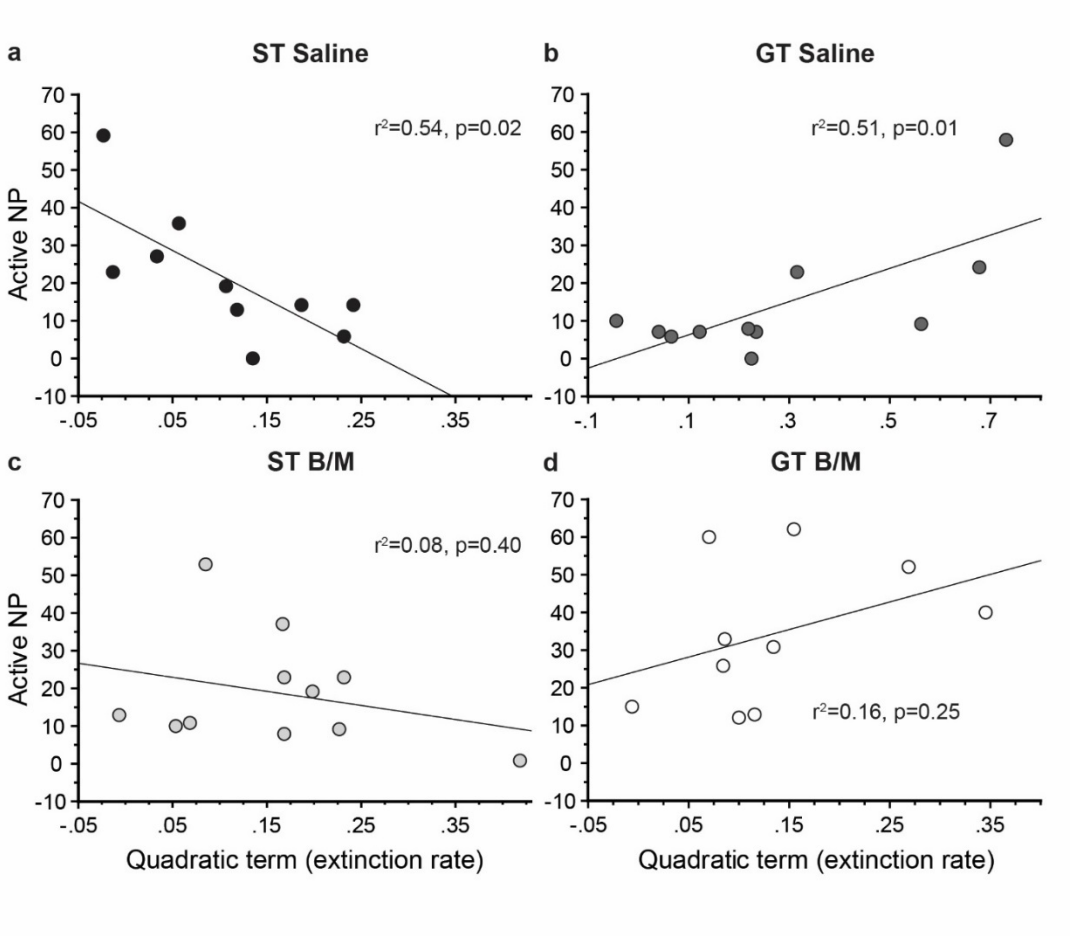

Fig. 2.6 Rate of extinction predicts cue-induced reinstatement of drug-seeking behavior in control groups. Scatterplots showing the relationship between the extinction rate and the number of pokes into the active port during cue-induced reinstatement of rats for each group (STs: Saline, n=10; B/M, n=11; GTs: Saline, n=11; B/M, n=10). (a) A faster extinction rate in STs resulted in a lower number of pokes made into the active port during reinstatement (p=0.02; r^2=0.54), (b) while a faster extinction rate in GTs resulted in a greater number of pokes into the active port during reinstatement (p=0.01; r^2=0.51). There were no significant relationships between extinction rate and pokes into the active port during reinstatement in (c) STs (p=0.40, r^2=0.08) or (d) GTs (p=0.25, r^2=0.16) with PVT inactivation.

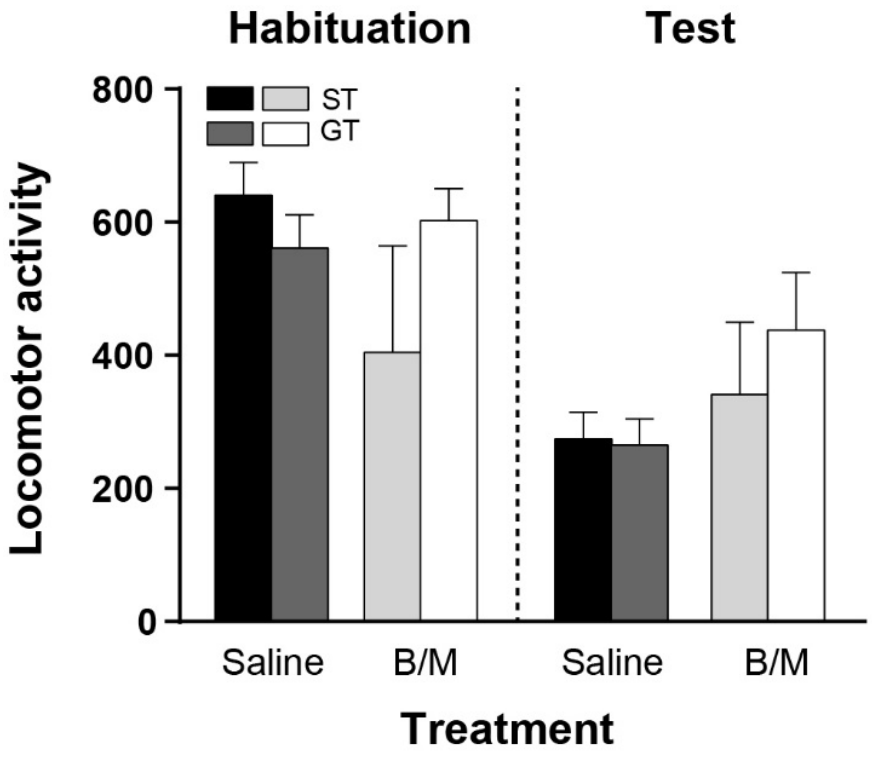

Fig. 2.7 Effects of PVT inactivation on locomotor activity. Mean + SEM for locomotor activity during habituation, followed by a test session before which rats received either saline or B/M into the PVT. There were no significant effects of phenotype or treatment for this measure, but all rats tended to decrease locomotor activity during the test session relative to habituation ($p<0.0001$). (ST Saline, n=5; ST B/M, n=4; GT Saline, n=6; GT B/M, n=7)

Chapter 3

Chemogenetic inhibition of a "top-down" cortico-thalamic circuit selectively attenuates cue-induced reinstatement of drug-seeking behavior in sign-trackers, but not goal-trackers

Abstract

Sign-tracking behavior has been shown to be reliant on "bottom-up" subcortical processing, whereas goal-tracking behavior is mediated by "top-down" cortical processing. However, sign-trackers (STs) and goal-trackers (GTs) both engage cortical inputs from the prelimbic cortex to the PVT in response to a food-paired cue (Haight et al. 2017). This led to a central hypothesis that the PrL-PVT pathway may mediate the predictive value of a reward-cue, but in the context of different neuromodulatory effects in STs and GTs. Data from our lab suggests that this pathway does in fact differentially mediate sign- and goal-tracking behavior. However, what remains unknown, and is the objective of this Chapter, is if the PrL-PVT pathway also differentially mediates drug-seeking behavior in STs and GTs. To address this, we used a dual-vector approach to selectively express inhibitory (G_i) DREADD (Designer Receptors Exclusively Activated by Designer Drugs) in the PrL-PVT pathway. Rats were characterized as STs or GTs based on their behavior during a Pavlovian conditioned approach task and then underwent 15 days of cocaine self-administration followed by 4 weeks of forced abstinence and subsequent extinction training. A test for cue-induced reinstatement followed, prior to which rats

received either vehicle or clozapine-N-oxide (CNO; 5 mg/kg) to activate the DREADD and inhibit the PrL-PVT pathway. After the cue-induced reinstatement test, rats underwent a brief period of forced abstinence followed by another period of extinction training, before undergoing a test for cocaine-primed reinstatement. Prior to cocaine-induced reinstatement, rats received the same treatment as previously administered (i.e. either vehicle or CNO), again to assess the effect of inhibiting the PrL-PVT pathway on drug-seeking behavior. Inhibition of the PrL-PVT circuit selectively attenuated cue-induced drug-seeking behavior in sign-trackers, without affecting the behavior of goal-trackers. Manipulation of this circuit did not affect cocaine-primed drug-seeking behavior in either phenotype. The PrL-PVT circuit, therefore, appears to play a specific role in mediating cue-induced drug-seeking behavior in sign-tracker rats.

Introduction

Relapse remains one of the biggest problems in the treatment of drug addiction, as cues in the environment (e.g. people, places, paraphernalia) associated with the drug-taking experience can elicit feelings of craving (Childress et al. 1988, Childress et al. 1993) and ultimately relapse (Shaham et al. 2003). Pavlovian learning processes contribute to these cue-reward associations. During Pavlovian learning, a cue is attributed with predictive value when it reliably precedes reward delivery. In addition to acquiring predictive value, Pavlovian cues can also acquire excessive motivational value and become "motivational magnets" (Berridge et al. 2009) via a process known as incentive salience attribution (Robinson and Berridge 1993). Once attributed with incentive salience, a cue can invigorate maladaptive behaviors such as drug-seeking and drug-taking behavior. However, there is considerable individual variation in the extent to which

a reward-cue is attributed with incentive value, and only for some individuals does a reward-cue attain inordinate control over behavior.

Using the sign-tracker (ST)/ goal-tracker (GT) animal model we are able to assess individual variation in cue-reward learning. In this model, rats undergo a Pavlovian conditioned approach training procedure by which rats can be classified into those that attribute predictive value to a reward-cue (GTs), and those that assign both predictive and incentive motivational value to a reward-cue (STs). In addition to showing individual variation in appetitive cue-reward learning, STs and GTs differ on a number of other addiction-related behaviors (Flagel et al. 2010, Saunders and Robinson 2010, Lovic et al. 2011, Saunders and Robinson 2011, Paolone et al. 2013, Saunders et al. 2013, Yager and Robinson 2013, Saunders et al. 2014, Yager et al. 2015, Pitchers et al. 2017a, Pitchers et al. 2017b). Specifically, relative to GTs, STs show greater drug-seeking behavior during tests for cocaine-primed (Saunders and Robinson 2011) and cue-induced reinstatement after limited drug experience and abstinence (2-4 weeks) (Saunders and Robinson 2010, Saunders et al. 2013). These differences in relapse propensity between STs and GTs support the long-standing belief that Pavlovian learning processes (Bolles 1972, Bindra 1978, Toates 1981, Stewart et al. 1984, Robinson and Berridge 1993), and more explicitly, incentive salience attribution (Robinson and Berridge 1993), underlie addiction. The ST/GT model, therefore, provides a means to elucidate the neurobiological mechanisms underlying these processes that contribute to relapse propensity.

STs and GTs have been shown to engage different neural circuitry in response to both food- and drug-associated cues, however the PVT appears to be a central node mediating both sign-and goal-tracking behavior (Haight et al. 2015). The PVT is a key component of the motive circuitry (Kalivas and Volkow 2005) and relays reward signals from cortical, limbic and motor

structures to regions in the striatum (Kelley et al. 2005). The PVT has garnered increasing attention recently for its role in mediating an array of motivated behaviors including fear learning (Li et al. 2014, Do-Monte et al. 2015, Penzo et al. 2015, Chen and Bi 2018), anxiety-related behaviors (Li et al. 2010, Barson and Leibowitz 2015, Beas et al. 2018), and reward learning (Flagel et al. 2011a, Haight et al. 2015, Yager et al. 2015, Do-Monte et al. 2017, Haight et al. 2017, Ong et al. 2017, Otis et al. 2017, Cheng et al. 2018, Choudhary et al. 2018, Haight et al. 2015). The PVT also exhibits changes in gene expression in response to drugs of abuse (Deutch et al. 1995, Deutch et al. 1998, Stephenson et al. 1999) and has been shown to mediate several addiction-like behaviors, including drug-seeking behavior during reinstatement. Specially, neuronal inhibition of the PVT attenuates drug-seeking behavior during tests of cocaine-primed (James et al. 2010), context-induced (Hamlin et al. 2009), cue-induced reinstatement (Matzeu et al. 2015, Matzeu et al. 2016). However, we recently reported that inactivation of the PVT prior to a test of cue-induced reinstatement robustly increases drug-seeking behavior in goal-trackers without affecting behavior in sign-trackers (Kuhn et al. 2018). These data suggest that the PVT mediates individual variation in relapse propensity by attenuating the incentive motivational value of the cocaine-cue only in GTs (Kuhn et al. 2018, see Chapter 2).

The mechanisms and circuitry by which the PVT exerts its effects on incentive motivational processes remains unclear. In response to a food-cue, GTs have been shown to engage cortico-thalamic circuitry, whereas STs engage hypothalamic-PVT-striatal pathways (Flagel et al. 2011a, Haight and Flagel 2014, Haight et al. 2017). These data suggest that GTs engage “top-down” processing whereas STs utilize “bottom-up” processing to guide behavior in response to a reward-cue. It is likely this “imbalance” in top-down versus bottom-up processing that contributes to sign- and goal-tracking behavior. Interestingly, however, when only those

cells that communicate with the PVT are assessed for cue-induced neuronal activity, both phenotypes engage afferents from the prelimbic cortex (PrL) to the PVT to the same extent (Haight et al. 2017). Thus, the PrL-PVT pathway could mediate the predictive value of a Pavlovian reward-cue (Haight et al. 2017), which is common to both phenotypes. Recent work from our lab shows that activation of the PrL-PVT pathway increases goal-tracking behavior in STs, presumably by overpowering the subcortically driven incentive motivational value of the reward cue (Campus et al. 2018). Conversely, inhibition of the PrL-PVT pathway increases sign-tracking behavior in GTs, allowing the incentive motivational value of the reward cue to be expressed (Campus et al. 2018). These data suggest that the PrL-PVT pathway acts to suppress the incentive motivational value of the reward cue, however the subcortical drive in STs overpowers this cortical drive resulting in the expression of incentive salience to reward-cues.

The role of cortical projections to the PVT have been assessed in other motivational behaviors, such as mediating fear memory (Do-Monte et al. 2015), conditioned reward-seeking during Pavlovian learning (Otis et al. 2017), and, recently, cue-induced reinstatement (Giannotti et al. 2018). However, what remains unknown is the role of the PrL-PVT pathway in mediating individual variation in the incentive motivational value of a cocaine-cue during cue-induced reinstatement. We also assessed the role of this pathway in individual variation in cocaine-primed reinstatement, as STs show greater cocaine-primed drug-seeking behavior compared to GTs (Saunders and Robinson 2011). To address this, we used an inhibitory DREADD (Designer Receptors Exclusively Activated by Designer Drugs) to specifically manipulate neurons in the PrL that project to the PVT. Rats were characterized as STs or GTs, and then underwent cocaine self-administration and extinction training. Prior to the test of cue-induced reinstatement, rats received an injection of either clozapine-N-oxide (CNO) to inhibit the PrL-PVT pathway, or a

vehicle injection. Next, rats underwent additional extinction training, followed by cocaine-primed reinstatement testing. Rats received the same treatment as that previously (CNO or vehicle) given prior to the reinstatement test. We hypothesized that the PrL-PVT pathway acts to suppress the incentive motivational value of the cocaine cue such that inhibiting this pathway prior to a test of cue-induced reinstatement would result in an overall disinhibition and expression of the incentive-motivational value of the cocaine cue. As our previous work has shown that allowing for the expression of the incentive motivational value of a cocaine-cue selectively increases cue-induced drug-seeking behavior in GTs (Kuhn et al. 2018), we hypothesized that inhibiting the PrL-PVT pathway would increase cue-induced drug-seeking behavior selectively in GTs (Kuhn et al. 2018). We also hypothesized that PrL-PVT inhibition would affect individual variation in cocaine-primed reinstatement of drug-seeking behavior, as the PVT has been shown to play a role in this form of reinstatement as well (James et al. 2010).

Methods

Subjects

A total of 180 male heterogeneous stock (HS) rats from a breeding colony at the Medical College of Wisconsin were initially screened to be used in this study. HS rats were originally bred from eight different strains of inbred rat lines and then outbred over several generations, resulting in more genetic and phenotypic diversity than most commonly used rat lines, such as Sprague-Dawley rats (Solberg Woods 2014). All experimental procedures used were approved by the University of Michigan Institutional Animal Care and Use Committee, and abided by the standards set in *The Guide for the Care and Use of Laboratory Animals: Eighth Edition*, published by the National Academy of Sciences and revised in 2011. Throughout the study, rats

had ad libitum access to food and water and were housed in a climate-controlled room with a 12-hour light: dark cycle (lights came on at either 06:00 h or 07:00 h depending upon daylight savings time). Upon arrival, rats were pair-housed until surgeries, after which they were single-housed for the remainder of the study to protect the catheters from possible damage from a cage mate. All behavioral training occurred during the light cycle, between 08:00 h and 18:00 h, with specific testing times for each procedure included below. Figure 3.1 shows the experimental timeline, with details for each procedure in the following sections.

Viruses

A dual vector DREADD (Designer Receptors Exclusively Activated by Designer Drugs) was used to selectively isolate and transiently inhibit neurons projecting from the prelimbic cortex (PrL) to the paraventricular nucleus of the thalamus (PVT). To accomplish this, a Cre-recombinase dependent viral vector flip-excision switch system was used. Using this approach, a Cre-dependent adeno-associated virus (AAV) containing the dual-floxed inhibitory DREADD (Addgene, pAAV-hSyn-DIO-hM4D(Gi)-mCherry, 1.9×10^{13} GC/ml, serotype 8) was injected into the PrL and traveled anterogradedly down the axon terminals to regions the PrL projects to. This viral construct contained the inverted G_i DREADD gene that requires the presence of Cre to flip it and be properly transcribed and translated into a functional receptor. The AAV-Cre (Addgene, AAV retrograde pmSyn1-EBFP-Cre, 7.6×10^{12} GC/ml) was then injected into the PVT and traveled retrogradedly from the axon terminal to the cell bodies that project to the PVT, including those in the PrL that contained the Gi DREADD gene. This approach, therefore, allows for the selective expression of DREADD in a specific pathway (e.g. the PrL-PVT pathway) (Carter et al. 2013, Boender et al. 2014, Wunsch et al. 2017, Yager et al. 2018).

Drugs

Cocaine HCl (Mallinckrodt, St. Louis, MO) dissolved in 0.9% sterile saline was used in these studies. Clozapine-N-oxide (CNO; Log number 13626-76) was obtained from the National Institute of Mental Health Chemical Synthesis and Drug Supply Program and used at a concentration of 5 mg/ml dissolved in 6% dimethyl sulfoxide (DMSO).

Surgical procedures

After arrival in our housing facilities, rats were allowed to acclimate to the housing room for a minimum of 5 days prior to surgery. Rats first underwent surgery to implant an indwelling jugular catheter for cocaine self-administration. Rats were anesthetized using ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and implanted with the jugular vein catheter as previously described (Crombag et al. 2000, Flagel et al. 2003). After the surgery was completed, rats were given a 5 ml injection (s.c.) of saline to minimize dehydration and facilitate recovery. To ensure sufficient recovery, rats underwent stereotaxic surgery 24 h to 48 h after catheterization surgery.

For stereotaxic surgery, rats were anesthetized using 5% isoflurane, and then maintained under 2% isoflurane for the duration of the surgery. Each stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) was outfitted with two digital manipulator arms (Stoelting, Wood Dale, IL). Rats were fitted into the ear bars, and then the scalp was cleaned with a series of Betadine solution (Purdue Products, Stamford, CT) and ethanol wipes prior to an incision being made to expose the skull. Bregma was determined, and the skull was then leveled such that lambda was within +/- 0.1 mm of the bregma coordinates. The G_i DREADD was injected

bilaterally into the PrL (relative to bregma: AP 3.0, ML: +/- 1.0, DV -4.0) over the course of 5 minutes at a rate of 200 nl/minute, for a total volume of 1 µL. These injections were made using a standard infusion pump (Pump 11 Elite, Harvard Apparatus) to depress a 5 µl Hamilton syringe with P50 tubing connecting it to a 31-gauge injector. The AAV-Cre was injected into the anterior PVT (relative to bregma: AP -2, ML -1, DV -5.4) and posterior PVT (relative to bregma: AP -3.0, ML -1.0, DV -5.5) over the course of 2 minutes at a rate of 50 nl/minute, for a total of 100 nl per injection site using a 1 µl Hamilton Microsyringe. Injections into the PVT were made at a 10° angle to the midline to avoid puncturing the superior sagittal sinus and causing unnecessary bleeding. At the completion of each injection, the injector was kept in place for an additional five minutes to allow the virus to diffuse away from the injector and into the tissue. At the conclusion of the surgery, surgical staples were used to bring the scalp back together.

Prior to the start of each surgery, and 24 h following surgery, an injection of 5 mg/ml of Carprofen was given as an analgesic. Additionally, rats received daily infusions i.v. of heparin (100 units/ml, 0.05 ml) and gentamicin sulfate (1 mg/ml, 0.05 ml) in order to decrease the chance of infection and maintain catheter patency until the conclusion of cocaine self-administration training. Following surgeries, rats were given a minimum of five days of recovery prior to the start of behavioral training. All surgical staples and sutures were removed within ten days of surgeries. Prior to the start of cocaine self-administration, catheter patency was checked using methohexital sodium diluted in sterile saline (10 mg/ml i.v., 0.1 ml). Rats were removed from the study due to lack of patency if the rat did not become ataxic within 10 seconds of infusion. Additionally, if rats stopped self-administering cocaine in the midst of the study, catheter patency was checked and rats were removed if the catheters were no longer patent.

Locomotor test

Rats were handled during the daily infusions of heparin and gentamicin sulfate during the surgery recovery period, which allowed them to acclimate to the experimenters. Rats underwent a locomotor test a minimum of 5 days following surgery. This test allowed us to characterize rats as "high-responders" or "low-responders" based on their locomotor response to novelty. Details of this test and discussion pertinent to these phenotypes are included in the Appendix of this Dissertation.

Pavlovian conditioned approach (PCA) training

Two days prior to the start of Pavlovian conditioned approach (PCA) training rats were given 45-mg banana-flavored grain pellets (about 25 pellets per rat; Bio-Serv, Flemington, NJ, USA) in their home cage to habituate them to the food reward used during training. Rats started PCA training the day following the locomotor test. Standard behavioral testing chambers (MED Associates, St. Albans, VT, USA; 20.5 x 24.1 cm floor area, 29.2 cm high) were used for PCA training. Chambers were situated in a sound-attenuating boxes fitted with a fan to provide constant background noise and air circulation. Within each chamber, a food magazine was situated 6-cm above the flooring and attached to a pellet dispenser. When the photo beam within the food magazine was broken a "contact" to the magazine was recorded. A retractable lever that illuminated upon presentation was situated to either the right or left of the food magazine and at the same height. In order for a lever "contact" to be registered and recorded, a minimum of 10-g of force had to be exerted. A white house light in the center of the opposite wall (1-cm from the top of the chamber) remained illuminated for the duration of each session.

Rats underwent two days of pre-training, with one session a day. During pre-training, the food magazine was baited with two banana-flavored grain pellets to direct the rats' attention to the location of reward delivery. After a 5-min acclimation period, the house light turned on and the session started, lasting approximately 12.5 minutes. During pre-training sessions, the lever remained retracted, and pellets were delivered to the food magazine on a variable interval 30-second schedule (range 0-30 seconds). Sessions consisted of 25 trials, with one pellet being delivered per trial. At the conclusion of the session, the house light turned off. The number of food magazine entries made and pellets remaining in the food magazine were recorded at the end of each session.

Following pre-training, rats underwent five PCA training sessions, with one session per day. As with pre-training, the start of each session was signaled with the house light turning on, and the conclusion by the house light turning off. During PCA training, the illuminated lever (conditioned stimulus, CS) entered the chamber for 8-sec, and immediately upon its retraction a banana-flavored grain pellet (unconditioned stimulus, US) was delivered to the food magazine. Each training session consisted of 25 lever-CS/ food-US trials on a variable interval 90-second schedule (range 30-150 seconds). Each session lasted approximately 40-min, and all training occurred between 10:00 h and 16:00 h.

For each PCA training sessions, the Med Associates software recorded the following information: (1) number of magazine contacts made during the 8-sec lever-CS presentation, (2) latency to the first magazine contact during lever-CS presentation, (3) number of lever-CS contacts, (4) latency to the first lever-CS contact, (5) the number of magazine entries made during the inter-trial interval (i.e. contacts made between lever-CS presentations). Using these measures, the PCA index was calculated for each rat and used to characterize rats into their

behavioral phenotypes based on their conditioned response (CR) made during training. The PCA index is a composite score that is based on response bias, latency and vigor of responding toward the lever-CS versus the food magazine (Meyer et al. 2012). The following formula is used to calculate the PCA index: [Probability Difference Score + Response Bias Score + (-Latency Difference Score)/3] (Meyer et al. 2012). Scores range from -1 to 1, with a score of -1 representing individuals with a CR always directed solely towards the food magazine during lever-CS presentation (e.g. goal-tracker, GT). Conversely, a score of 1 represents individuals with a CR always directed toward the lever-CS upon its presentation (e.g. sign-tracker, ST). The PCA index for session 4 and 5 were averaged to compute the final PCA index used to characterize rats into their respective behavioral phenotypes. For these studies, rats were characterized as STs if they had a PCA score between 0.3 and 1, GTs between -0.3 and -1, and intermediate rats (IN), individuals who vacillate between the two CRs, between -0.29 and 0.29.

Food self-administration

Following PCA training, rats had a 24-hour break during which they remained in the colony room undisturbed before starting food self-administration training. Rats underwent this training in order to learn to make an operant response for a reward, thus decreasing attrition during cocaine self-administration due to not acquiring the instrumental response. Food self-administration took place in the same testing chambers as PCA training, however the chambers were reconfigured such that the lever was removed, and two nose ports were put on either side of the food magazine. In order to minimize location bias, the “active” port was on the opposite side of the food magazine as the lever-CS was during PCA training. The house light and a discrete cue light within the active port turned on one minute after the program was started. The cue light

remained on until the first poke was made into the active port in order to direct the rat's attention to the port. Pokes into the active port resulted in the delivery of one 45-mg banana flavored grain pellet (FR1 schedule of reinforcement), the same reward used during PCA training, as well as presentation of the cue-light inside the port for 20 seconds. Additional pokes made into the active port during the 20-sec cue-light presentation period were recorded, but were without consequence. During cue-light presentation, the house light also turned off. Pokes into the inactive port were recorded, but without consequence. Rats underwent four days of food self-administration testing, with one session per day. Sessions were terminated after rats had acquired 25 pellets, or after 30-min. All training occurred between 10:00 h and 14:00 h.

Cocaine self-administration

At the conclusion of food self-administration, rats were left undisturbed in the colony room for 24 hours prior to the start of cocaine self-administration. Testing chambers remained in the same configuration as food self-administration, with the exception that the food magazine and pellet dispenser were removed. The house light and cue light in the active port turned on one minute after the session started. In contrast to food self-administration, the cue light remained on for 20-sec at the start of the session, during which time a poke into the active port could result in an infusion of cocaine. Throughout the session, pokes into the active port (FR1 schedule of reinforcement) resulted in the presentation of the cue light for 20-sec and a 0.5 mg/kg infusion of cocaine (Mallinckrodt, St. Louis, MO) diluted in 0.9% sterile saline, delivered in 25 μl over 1.6 seconds. Additional pokes into the active port during the 20-sec cue light presentation were recorded, but without consequence. Pokes made into the inactive port were recorded, but also without consequence. Sessions terminated after 3 hours, or once the rat met the infusion criterion

(IC) for that session. An IC is the maximum number of infusions a rat can receive during a given session (Saunders and Robinson 2010, Saunders and Robinson 2011, Saunders et al. 2013, Flagel et al. 2016). Infusion criterion ensure that rats receive the same number of infusions each training session, as well as the same number of cocaine cue-light pairings. Rats underwent one cocaine self-administration training session per day for 15 consecutive days between 8:00 h and 18:00 h. The following schedule was used: three days at IC5, four days at IC10, 3 days at IC20, and 5 days at IC45. During the IC45 sessions, the cocaine infusion dose was decreased to 0.2 mg/kg/infusion to enhance drug-seeking behavior (Saunders and Robinson 2010). In order to move to the next IC and remain in the study, rats had to meet the IC for two consecutive sessions and maintain catheter patency. At the conclusion of training, rats underwent a 28-day period of forced abstinence where they were left undisturbed in the colony room. Compared to shorter time periods, 28 days of forced abstinence has been showed to lead to an increase in drug-seeking behavior during tests for reinstatement (Grimm et al. 2001).

Extinction training 1

Testing chambers remained in the same configuration as that during cocaine self-administration, however pokes into both the active and inactive nose port were recorded, but without consequence (i.e. pokes into the active port did not result in cue-light presentation or drug delivery). Sessions lasted for 2 hours, and rats underwent one session per day for a total of 13 sessions. Rats had to make less than ten pokes into the active port for two consecutive sessions in order to be eligible to move to the test for cue-induced reinstatement, or they were eliminated from the study. All training took place between 09:00 h and 18:00 h. After the last training session, rats were moved to an adjacent testing room and given an i.p. injection of 6%

DMSO in 0.9% sterile saline (CNO vehicle) to habituate them to the injections that would occur prior to the reinstatement test.

Cue-induced reinstatement test

Rats underwent a test for cue-induced reinstatement the day following the last extinction training session (i.e. session 13). Rats were counterbalanced into treatment groups based on behavior during PCA training (lever- and magazine-directed behavior, PCA index), locomotor index (cumulative locomotor movements), and behavior during cocaine self-administration and extinction training (nose pokes made into the active and inactive ports). Twenty-five minutes prior to the test, rats received either an injection of CNO (5 mg/kg i.p.) to activate the G_i DREADDs or vehicle (6% DMSO in 0.9% sterile saline) in an adjacent testing room. After the rats were placed in the testing chambers, one minute elapsed prior to illumination of the house light. The cue light in the active port also came on at the session start, and remained on for 20 seconds, as it did during the cocaine self-administration procedure. Pokes into the active port were recorded and resulted in presentation of the cue light for 5-sec, but no cocaine infusion, while pokes into the inactive port were recorded but without consequence. The test lasted for 2 h and occurred between 10:00 h and 17:00 h.

Forced abstinence and extinction training 2

After the test for cue-induced reinstatement, rats underwent five days of forced abstinence followed by eight days of extinction training as previously described prior to the test for cocaine-primed reinstatement. Extinction training was performed again to ensure that all rats were drug-seeking at the same rate prior to the test for cocaine-primed reinstatement. Rats had to

make less than ten pokes into the active port for two consecutive sessions in order to be eligible to move to the test for cocaine-primed reinstatement, or they were eliminated from further testing. After the last extinction session, rats received an i.p. injection of 6% DMSO in 0.9% sterile saline (CNO vehicle) in an adjacent testing room to habituate them to the test day procedure. Cue-induced reinstatement always preceded cocaine-primed reinstatement in this experiment in order to keep the experience of each rat as consistent as possible.

Cocaine-induced reinstatement test

The day after the last extinction training session (i.e. session 8), all rats underwent a test for cocaine-induced reinstatement. As with the cue-induced reinstatement test, rats received the same treatment (i.e. CNO or vehicle) 25-min prior to the session in an adjacent testing room as. Before being put into the testing chamber, all rats received a 15 mg/kg (i.p.) injection of cocaine. This dose of cocaine was chosen as it has previously been shown to result in ST/GT differences in drug-seeking behavior during a test for cocaine-induced reinstatement (Saunders and Robinson 2011). The house light came on one minute after the session was initiated, but in contrast to the cue-induced reinstatement test the cue-light did not come on at any point during the test session. Pokes into the active and inactive port were recorded, but without consequence (i.e. no cue-light presentation when pokes were made into the active port). The session lasted for 2 h and occurred between 10:00 h and 17:00 h.

Histology

Once testing was complete, all rats were anesthetized with ketamine (90 mg/kg. i.p.) and xylazine (10 mg/kg, i.p.) and underwent transcardial perfusion. Rats were first perfused with

0.9% saline followed by 4% formaldehyde (pH= 4.7) at 4°C. After extraction, brains remained in formaldehyde for a 24 h period. Brains were then moved to graduated sucrose solutions (10%, 20% then 30% sucrose in phosphate buffer, pH= 7.4, 4°C) every 24 hours for 3 days for cryoprotection. Following cryoprotection, brains were encased in Tissue-Plus O.C.T. cryoprotectant (Fisher HealthCare, Houston, TX) and frozen using dry ice. They were then sliced at a thickness of 30 µm on a cryostat and underwent free-floating immunohistochemistry (IHC) for mCherry (protein fused to DREADD) to assess hM4D(G_i) DREADD expression. All procedures occurred at room temperature and on a lab shaker on a low speed. Slices were washed in 0.1 M phosphate-buffered saline (PBS, pH= 7.3-7.4) five times (5-min each wash) and then blocked in 1% hydrogen peroxide in PBS solution for 10-min. Slices were then washed in PBS (4 washes) and blocked in 0.4% Triton-X and 2.5% Normal Donkey Serum (Jackson ImmunoResearch, West Grove, PA) diluted in PBS for one hour, and then incubated overnight in a PBS solution containing 0.4% Triton-X and 1% Normal Donkey Serum with rabbit anti-mCherry primary antibody (Abcam, Cambridge, MA) at a 1:30,000 dilution. The next day, slices were washed three times in PBS and incubated for one hour in a PBS solution containing biotinylated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, West Grove, PA) at a 1:500 dilution, 0.4% Triton-X, and 1% Normal Donkey Serum. After another series of three PBS washes, slices were incubated for one hour in ABC-elite (Vector Laboratories, Burlingame, CA) diluted at 1:1000 in PBS, and then underwent three PBS washes. In order to view DREADD expression, slices were then incubated in a 0.1M sodium phosphate-buffered solution containing 0.02% 3, 3’ diaminobenzidine tetrahyrochloride (DAB; Sigma Aldrich, St. Louis, MO) and 0.012% hydrogen peroxide. Incubation lasted for 8-min, and then slices were rinsed three times in PBS and stored at 4°C until being mounted. DAB staining creates a brown residue at the

location of DREADD expression, allowing for subsequent visualization. After mounting, brains were dehydrated in ethanol solution, washed in xylene and coverslipped with Permount (Fisher Scientific, Fair Lawns, NJ). Using a Leica DM1000 light microscope (Buffalo Grove, IL), DREADD expression in the PrL, aPVT and pPVT were qualitatively scored by two researchers who were blind to group assignments. DREADD expression scores ranged from 0 to 3, and were based on localization and strength of the expression. In order to be included in final analysis (scores of 1-3), DREADD expression had to be localized to the PrL (relative to bregma: AP 5-2.50), aPVT (relative to bregma: AP -1.20--2.50) and pPVT (relative to bregma: AP -2.51--3.50), and had to be easily visible in the microscope. A score of 1 indicated weak expression, whereas a score of 3 indicated strong expression. Rats with a score of zero (e.g. inaccurate injection, lack of expression in a region, etc.) were not included in final analyses.

C-fos protein expression in response to test day treatment (CNO or vehicle) was analyzed in a subset of rats (GT VEH: 10, GT CNO: 8, ST VEH: 3, ST CNO: 6) to verify that the G_i DREADDs were in fact affecting the activity in the PrL-PVT pathway. Eighty minutes prior to perfusions, rats were injected (i.p.) with either 5.0 mg/kg CNO or vehicle (same as test day treatment) and left in the colony room. This time period was chosen as c-fos protein expression has been shown to peak 60-90-min after cellular activation (Sonnenberg et al. 1989). Brains from rats with good DREADD expression underwent free-floating IHC for co-expression of c-fos protein and DREADD expression. The IHC for c-fos protein was performed first, and all procedures are the same as previously discussed above with the following exceptions: a goat anti-cfos primary antibody (Santa Cruz Biotechnology, Dallas, TX) diluted at 1:1000 and a biotinylated donkey anti-goat secondary antibody (Jackson ImmunoResearch, West Grove, PA) diluted at 1:500 were used. Additionally, nickel sulfate (0.08%) was added to the DAB solution

so that c-fos protein expression would have a black, and not brown residue, allowing for contrast between the two stains. Immediately following the IHC for c-fos protein, the IHC for DREADD expression was completed as described above. All mounting, coverslipping, and visualization procedures are the same as outlined in the paragraph above. All of the tissue was processed using IHC for co-expression of c-fos and DREADD, but only c-fos protein quantified in the PVT are included in this Chapter.

To assess the effects of PrL-PVT inhibition on activity in the PVT, c-fos protein expression was quantified in the PVT. One PVT section in the anterior (relative to Bregma: -2.00), middle (relative to Bregma: AP -2.50), and posterior (relative to Bregma: AP -3.00) subregions was analyzed using ImageJ (National Institute of Health, Bethesda, MD). The number of c-fos positive cells and density of expression (cells per square mm) were determined using the ITCN (image-based tool for counting nuclei) plugin from ImageJ.

Video analysis: Cocaine-primed reinstatement test

Both stereotyped and non-stereotyped behaviors during the cocaine-primed reinstatement test for each rat was quantified. Quantified behavior was selected due its high presence shown by several rats when videos were randomly sampled for screening. Stereotyped behaviors include: circling, head movements but no port entry, head movements and port entry. Non-stereotyped behaviors include: immobility, orientation toward the active port without movement toward it, approach to the active port without port entry. A more detailed description of the criteria for each behavior can be found in Table 3.1. Behavior was analyzed for 30-sec every ten minutes for the first hour of the reinstatement test, as this is when peak drug-seeking behavior occurred. The percentage of time (min) rats spent exhibiting each behavior across the six bins was quantified.

Statistical analyses: Behavior

Data from PCA training, cocaine self-administration, and extinction training sessions were analyzed using a linear mixed-effects model. For each data set, the lowest Akaike's information criterion was used to determine the best covariance structure. The effect of Session, Phenotype and Treatment were assessed for lever-directed behavior using the following variables: probability to contact the lever, number of lever contacts, and latency to contact the lever upon presentation were the dependent variables. The same independent variables were used to assess magazine-directed behavior using the following dependent variables: probability to contact the magazine, number of magazine contacts, and the latency to approach the magazine during lever-CS presentation. The effect of IC, Port (active or inactive), Phenotype (ST or GT) and Treatment (VEH or CNO) were assessed for the number of nose pokes made across infusion criterion. Nose pokes made during extinction training were analyzed for the effects of Session, Port, Phenotype and Treatment. The relationship between the rate of extinction and drug-seeking behavior during the subsequent reinstatement test was also assessed. To do so, a quadratic regression model was fit to the extinction training curve for each rat, and then the quadratic term was regressed onto the number of nose pokes made into the active port during the reinstatement test. All of these analyses used SPSS Statistics Program (Statistical Package for the Social Sciences), version 22 (IBM, Armok, NY).

Nose pokes made during the reinstatement tests were analyzed using a three-way ANOVA, with Phenotype, Treatment and Port as independent variables. To compare drug-seeking behavior (i.e. pokes into the active-inactive port) between the last extinction training session and the reinstatement test session, a repeated measure ANOVA was used. Data was

analyzed for the effect of Session (Extinction vs Reinstatement), Phenotype and Treatment. A repeated measures ANOVA was used to assess the effect of Phenotype and Treatment on the percent of time engaged in stereotyped behavior versus non-stereotyped (Category) behavior during the cocaine-primed reinstatement test. Correlations between nose pokes made into the active port during the tests for reinstatement and other behavioral measures throughout training were also conducted using StatView.

Statistical analyses: Immunohistochemistry

The average density of c-fos positive cells between the anterior, middle and posterior PVT were analyzed using a repeated measures ANOVA with Phenotype, Treatment and Region (anterior, middle, posterior) as the independent variables. When no significant effects were detected, data were collapsed across a given variable for further analyses. Thus, average density of c-fos positive cells collapsed across the entire rostral-caudal axis of the PVT were assessed using a two-way ANOVA, with Phenotype and Treatment as the independent variables. In addition, rats in the vehicle group, regardless of phenotype, were collapsed into a single group. An unpaired t-test was then used to assess differences between CNO-treated STs and the vehicle group, and CNO-treated GTs and the vehicle group. To further assess if PrL-PVT inhibition affected c-fos density in the PVT of STs and GTs, the percent of c-fos density relative to all vehicle control rats (i.e. “baseline”) was calculated for each phenotype. A single sample t-test with the hypothesized value set to 100% (i.e. baseline) was then performed. Following this analysis, an unpaired t-test was performed on the same data to determine if PrL-PVT inhibition differentially affected PVT c-fos density relative to baseline in STs versus GTs. All ANOVAs were performed using StatView, version 5.0 (SAS Institute Inc., Cary, NC, USA).

When significant main effects or interactions were detected, post-hoc analyses were conducted using Bonferroni tests to correct for multiple comparisons. For all tests, statistical significance was set at $p<0.05$. Outliers for the cocaine-primed reinstatement test and video analysis were identified using the Grubb's outlier test (alpha=0.05).

Results

Subjects

Seven rats died during catheterization or stereotaxic surgery, prior to training. Three rats were eliminated from the study following PCA training as they did not acquire the task (i.e. did not approach the lever-CS or food magazine during training). One rat was sacrificed at the conclusion of food self-administration training due to health complications. Additional rats were removed from the study if they did not meet the IC during self-administration training (n= 51 (ST: 4/36; GT: 29/96; IN: 17/38) or due to loss of catheter patency, n= 1 (ST)).

Histology

Only rats that successfully completed all training and exhibited accurate DREADD expression as previously described were included in final analysis (ST VEH, n=9; ST CNO, n=6; GT VEH, n=16, GT CNO, n=15). There was an insufficient number of IN rats in each group (n=5/group) and so IN rats were excluded from final analyses. A subset of these rats underwent immunohistochemistry for c-fos protein and DREADD expression (GT VEH: 10, GT CNO: 8, ST VEH: 3, ST CNO: 6).

PCA behavior

There was an Effect of Session ($p<0.0001$), Phenotype ($p<0.0001$) and Session by Phenotype interaction ($p<0.0001$) for each lever-directed variable (Fig. 3.2a-c). Compared to GTs, sign-tracking rats showed a greater probability to contact the lever ($p<0.0001$), greater number of contacts made with the lever during presentation ($p<0.0001$), and lower latency to contact the lever upon presentation ($p<0.0001$) (Fig. 3.2 a-c). Post-hoc analyses revealed that STs differed from GTs on all three measures across the five sessions ($p<0.0001$ for all). For magazine-directed behavior there was a significant Effect of Session ($p<0.005$), Phenotype ($p<0.0001$) and a Session by Phenotype interaction ($p<0.0001$) for all variables (Fig. 3.2d-f). Compared to STs, GTs showed a greater probability to contact the food magazine ($p<0.0001$), greater number of food contacts ($p<0.001$), and lower latency to approach the food magazine upon lever-CS presentation ($p<0.0001$) (Fig. 3.2d-f). Post-hoc analyses showed that GTs differed from STs for the probability to approach the food magazine, food magazine contacts and latency to approach the food magazine on sessions two through five ($p<0.0001$) (Fig. 3.2d-f). There were no Effects of Treatment between or within phenotypes for lever- or magazine-directed behavior.

STs and GTs acquire cocaine self-administration at the same rate

All rats increased nose pokes into the ports throughout the course of training (Effect of IC, $F_{3,85}=84.88$, $p<0.001$), and differentiated between the active and inactive port (Effect of Port, $F_{1,85}=215.68$, $p<0.001$) (Fig. 3.3a). An interaction between IC and Port ($F_{3,85}=63.32$, $p<0.0001$) showed that rats responded more in the active port across IC (Effect of IC, $F_{3,85}=146.825$, $p<0.001$), but nose pokes made into inactive port did not change across IC (Effect of IC, $F_{3,85}=1.38$, $p=0.25$). In fact, rats made more nose pokes into the active port as compared to the inactive port at each IC (Effect of Port, $p<0.0001$) (Fig. 3.3a). There was no Effect of Phenotype

($F_{1,85}$=0.60, p=0.44) or Treatment ($F_{1,85}$=0.80, p=0.78) in the acquisition of cocaine self-administration, but this was to be expected as prior studies using the IC paradigm have shown that STs and GTs don't differ in acquisition of cocaine self-administration, and the Treatment designation refers to the Treatment that *will be* administered prior to reinstatement. However, unlike previous experiments, more GTs (30%) were excluded from the study due to not meeting IC requirements compared to STs (11%). This suggests that while behavior did not differ between rats that did acquire cocaine self-administration, STs are more likely to acquire self-administration compared to GTs, at least in this study.

STs and GTs extinguish drug-seeking behavior at the same rate

STs and GTs did not differ from one another throughout extinction (Effect of Phenotype, $F_{1,84}$=2.50, p=0.12), and behavior was not different between assigned Treatment groups (Effect of Treatment, $F_{1,84}$=0.12, p=0.73) (Fig. 3.3b). There was a significant Effect of Port (Effect of Port, $F_{1,84}$=36.77, p<0.0001) demonstrating that rats differentiated between the active and inactive port, and a significant Effect of Session (Effect of Session, $F_{12,84}$=24.04, p<0.0001) suggesting that responding changed over the course of training (Fig. 3.3b). A significant interaction between Session and Port ($F_{12,84}$=5.61, p<0.0001), however, revealed that responding between the active and inactive ports did not differ later in training (Session 9 (p=0.18), Session 10 (p=0.81), Session 11 (p=0.07)) (Fig. 3.3b).

Inhibition of the PrL-PVT pathway selectively decreases cue-induced drug-seeking behavior in STs

All rats differentiated between the active and inactive port (Effect of Port, $F_{1,84}$=41.13, p<0.001) during this test, making more nose pokes into the active port compared to the inactive port (active vs. inactive; p<0.03 for each group: ST VEH, ST CNO, GT VEH, GT CNO) (Fig. 3.4a). There was a significant Effect of Phenotype ($F_{1,84}$=9.85, p=0.002), Effect of Treatment ($F_{1,84}$=6.44, p=0.01) and a Phenotype x Treatment x Port interaction ($F_{1,84}$=6.14, p=0.02), suggesting that responses made at each port was dependent on both phenotype and whether or not the PrL-PVT circuit was inhibited. Post-hoc analyses revealed that rats in the ST VEH group made more nose pokes into the active port compared to GTs in the vehicle group (p<0.0001) or GT CNO group (p=0.01) (Fig. 3.4a). These results support previous findings that, relative to GTs, STs show greater drug-seeking behavior during a test for cue-induced reinstatement (Saunders and Robinson 2010, Saunders et al. 2013). Inhibition of the PrL-PVT pathway in GTs did not affect nose pokes into either the active (p=0.77) or inactive port (p=0.88) compared to the GT vehicle group. However, inhibition of the PrL-PVT pathway in STs led to a decrease in nose pokes made into the active port (p<0.001), but not inactive port (p=0.88), compared to the ST vehicle group (Fig. 3.4a). Additionally, PrL-PVT inhibition renders STs similar to GTs in the vehicle (p=0.70) and CNO group (p=0.54) (Fig. 3.4a). These data suggest the PrL-PVT pathway mediates the incentive motivational value attributed to the cocaine-cue selectively in STs. Additionally, correlations between PCA and index score and nose pokes made into the active port during cue-induced reinstatement.

To compare responding during the cue-induced reinstatement test to that during the last extinction session, we examined the effects of Phenotype (ST or GT) and Treatment (VEH or CNO) on the number of nose pokes in the active port minus those in the inactive port (i.e. active – inactive nose pokes; dependent variable). All rats showed an increase in drug-seeking behavior

during the test for cue-induced reinstatement compared to the last extinction training session (Effect of Session, $F_{1,42}$=34.87, p<0.0001) (Fig. 3.4b). There was a significant Effect of Phenotype ($F_{1,42}$=10.07, p=0.003), Effect of Treatment ($F_{1,42}$=9.37, p=0.004) and a Phenotype x Treatment x Session interaction ($F_{1,42}$=4.55, p=0.04), indicating that responding was differentially affected between sessions in a phenotype- and treatment-dependent manner. Drug-seeking behavior did not differ based on phenotype or treatment during the last day of extinction training (Effect of Phenotype, $F_{1,42}$=0.59, p=0.49; Effect of Treatment, $F_{1,42}$=0.37, p=0.55). For the reinstatement session, however, there was a Phenotype x Treatment interaction ($F_{1,42}$=6.85, p=0.01). Post hoc analyses showed that rats in the ST vehicle group showed more drug-seeking behavior (active-inactive nose pokes) compared to rats in the GT vehicle (p=0.007) or GT CNO group (p=0.005) (Fig. 3.4b). However, inhibition of the PrL-PVT pathway in STs during the reinstatement test decreased drug-seeking behavior compared to STs in the vehicle group (p<0.05), such that STs that received CNO did not significantly differ from the GT vehicle (p=0.94) or GT CNO group (p=0.62) (Fig. 3.4b). These findings support those above, suggesting that the PrL-PVT circuit selectively mediates cue-induced reinstatement in STs, who are inherently prone to this behavior. Lastly, it should be noted that there were no significant correlations between nose pokes made into the active port during the test for cue-induced reinstatement and PCA index score (ST VEH, r^2=0.55, p= 0.09; ST CNO, r^2=0.02, p= 0.70; GT VEH, r^2=0.02, p=0.61; GT CNO, r^2=0.05, p= 0.43). Thus, the degree to which one sign- or goal-tracks does not appear to predict cue-induced reinstatement of drug-seeking behavior.

Rats do not differ in rate of extinction training after cue-induced reinstatement

Similar to the first phase of extinction training, rats decreased drug-seeking behavior across sessions (Effect of Sessions, $F_{7,135}$=6.17, p<0.0001), and differentiated between the active and inactive port (Effect of Port, $F_{1,87}$=8.26, p<0.001) (Fig. 3.5a). Drug-seeking behavior did not differ between phenotype (Effect of Phenotype, $F_{1,87}$=1.54, p=0.22) or test day treatment (Effect of Treatment, $F_{1,87}$=0.26, p=0.61). In contrast to the first phase of extinction training, there was not a significant Session by Port interaction present, likely because pokes made into the active port started out much lower.

Inhibition of the PrL-PVT pathway does not affect cocaine-induced reinstatement of drug-seeking behavior

Prior to the start of the cocaine-induced reinstatement test, rat received a 15 mg/kg injection (i.p.) of cocaine. One rat (GT VEH) was excluded from statistical analyses using the Grubb's outlier test. Through video analyses, it was evident that this rat was exhibiting cocaine-induced stereotyped behavior manifested as pokes into the active port.

All rats made more pokes into the active port compared to the inactive port during the test (Effect of Port, $F_{1,82}$=25.70, p<0.0001) (Fig. 3.5b). There was no Effect of Phenotype ($F_{1,82}$=1.24, p=0.27) or Treatment ($F_{1,82}$=0.74, p=0.39). There was no Phenotype by Port interaction ($F_{1,82}$=0.04, p=0.84), suggesting unlike previous findings, STs and GTs did not differ in cocaine-induced drug-seeking behavior (Saunders and Robinson 2011) (Fig. 3.5b). There was also not a Phenotype x Treatment x Port interaction ($F_{1,82}$=0.12, p=0.73) present, suggesting that PrL-PVT inhibition did not affect cue-induced reinstatement.

Drug-seeking behavior (active-inactive nose pokes) between the last extinction session and the test for cocaine-induced reinstatement was analyzed as a means to directly compare

responses between the two sessions. All rats showed greater drug-seeking behavior during the test for cocaine-induced reinstatement compared to the last extinction training session (Effect of Session, $F_{1,41}$=37.41, p<0.0001) (Fig. 3.5c). However, there was not a significant Effect of Treatment (Effect of Treatment, $F_{1,41}$=0.01, p=0.93), Effect of Phenotype (Effect of Phenotype, $F_{1,41}$=0.05, p=0.82), nor any significant interactions present.

In an attempt to account for the variance in drug-seeking behavior seen during this test, correlations between the number of nose pokes made into the active port and other behaviors throughout the study were assessed. There were no significant correlations between nose pokes made into the active port during the tests for cue-induced reinstatement and cocaine-primed reinstatement (ST VEH, r^2=0.04, p= 0.70; ST CNO, r^2=0.04, p= 0.60; GT VEH, r^2=0.23, p=0.07; GT CNO, r^2<0.001, p= 0.96); nor was there a significant correlation between PCA index score and cocaine-primed drug-seeking behavior (ST VEH, r^2=0.51, p= 0.11; ST CNO, r^2=0.001, p= 0.95; GT VEH, r^2=0.11, p=0.23; GT CNO, r^2=0.10, p= 0.26).

Cocaine-induced stereotyped behaviors during cocaine primed-reinstatement

Stereotyped behaviors during cocaine-primed reinstatement were assessed in a subset of rats (ST VEH, n=4; ST CNO, n=3; GT VEH, n=10; GT CNO, n=9). Rats spent more time exhibiting “non-stereotyped behaviors” (e.g. active port orientation/ approach, immobility) compared to “stereotyped behaviors” (e.g. head movements, circling) (Effect of Category, $F_{1,22}$=8.13, p<0.001) (Fig 3.6). On average, rats spent ~5-10% percent of the time exhibiting stereotyped behaviors. STs and GTs did not differ on this measure (Effect of Phenotype, $F_{1,22}$<0.0001, p=0.99) and treatment did not affect cocaine-induced stereotypy (Effect of Treatment, $F_{1,22}$=0.04, p=0.84) (Fig 3.6). These data suggest that at least some animals exhibited

stereotypy, which may have interfered with cocaine-induced reinstatement measures; but the small sample size and lack of significant treatment and phenotype effects warrants further investigation in this regard.

Rate of extinction and reinstatement of drug-seeking behavior

There was not a significant relationship present between the rate of extinction (extinction 1; i.e., the change in responding in the active nose port across sessions) and the number of nose pokes made into the active port during the test for cue-induced reinstatement (ST VEH, r^2=0.28, p=0.29; ST CNO, r^2=0.28, p=0.17; GT VEH, r^2=0.01, p=0.79; GT CNO, r^2=0.05, p=0.45). This suggests that the rate by which rats decreased nose pokes made into the active port during extinction training did not correlate with cue-induced reinstatement of drug-seeking behavior. There was also no relationship present between the rate of extinction (extinction 2) and the number of nose pokes made into the active port during cocaine-primed reinstatement (ST VEH, r^2=0.41, p=0.17; ST CNO, r^2=0.02, p=0.73; GT VEH, r^2=0.02, p=0.67; GT CNO, r^2=0.20, p=0.10), suggesting that variation in drug-seeking behavior seen during this test is not due to differences in extinction rate.

PVT c-fos expression

Density of c-fos protein expression in the PVT was initially analyzed across sub-regions (anterior, middle and posterior) of the PVT, but there was not a significant effect of Region ($F_{2,40}$=1.31, p=0.28) nor a significant Phenotype x Treatment x Region interaction ($F_{2,40}$=0.37, p=0.70), so subsequent analyses focused on the average density of c-fos expression across the rostral-caudal axis of the PVT. When density of c-fos expression across the entire PVT was

averaged, there was a significant Phenotype x Treatment interaction ($F_{1,23}$= 4.29, $p<0.05$), and post-hoc analyses showed that inhibition of the PrL-PVT differentially affected average c-fos density in STs relative to GTs (p=0.002). However, when compared to their respective vehicle-treated controls, PrL-PVT inhibition did not affect average c-fos density in STs (p=0.19) or GTs (p=0.10). Because there was a not a significant difference in average c-fos density between the vehicle-treated groups (p=0.49), additional analyses were conducted with a single vehicle group collapsed across phenotypes. Consistent with the data reported above, there was not a significant effect of CNO treatment on average c-fos density in STs (t(17)=0.71, p=0.49) or GTs (t(19)=-1.50, p=0.15) relative to the collapsed vehicle group. To further assess whether PrL-PVT inhibition differentially affected c-fos expression in STs and GTs, the percent of average c-fos density relative to baseline (i.e. collapsed vehicle control group) was considered as the dependent variable. Using this metric, PrL-PVT inhibition resulted in greater PVT c-fos density in STs compared to GTs relative to baseline (t(12)=-4.10, $p<0.002$; Fig 3.7b), which is also consistent with the results reported above using the raw data. Relative to baseline (i.e. 100%), inhibition of the PrL-PVT did not significantly affect PVT c-fos density in STs (t(5)=1.36, p=0.23), but did decrease PVT c-fos density in GTs (t(7)=-9.01, $p<0.001$) (Fig. 3.7b). These results support the notion that the PrL-PVT pathway differentially mediates downstream activity STs and GTs.

Discussion

In this study, we assessed the role of cortical projections from the prelimbic cortex (PrL) to the paraventricular nucleus of the thalamus (PVT) on individual variation in cue-induced and cocaine-primed reinstatement of drug-seeking behavior. Using the ST/ GT model, we are able to capture individual variation in the incentive motivational properties of a reward cue (Robinson

and Flagel 2009), as well as individual variation in several addiction-related behaviors (Flagel et al. 2010, Saunders and Robinson 2010, Lovic et al. 2011, Paolone et al. 2013, Saunders et al. 2014, Pitchers et al. 2017a, Sarter and Phillips 2018). This is especially pertinent, as STs show greater drug-seeking behavior during tests of cue-induced (Saunders and Robinson 2011, Saunders et al. 2013) and cocaine-primed reinstatement (Saunders and Robinson 2010) relative to GTs. Thus, this model allows us to assess individual variation in relapse propensity and the underlying neurobiological mechanisms that mediate these differences.

The PVT is a central node that mediates individual variation in incentive salience attribution (Haight et al. 2015), as well as cue-induced reinstatement of drug-seeking behavior (Kuhn et al. 2018). The projections from the PrL to the PVT have been implicated in mediating cue-reward learning (Do-Monte et al. 2015, Otis et al. 2017, Giannotti et al. 2018), and specifically incentive salience attribution (Campus et al. 2018). In this study, we assessed the effects of inhibiting the PrL-PVT pathway on individual variation in cue-induced and cocaine-primed reinstatement in STs and GTs. During the test for cue-induced reinstatement, nose pokes made into the port that previously resulted in cocaine delivery now only resulted in presentation of the cocaine-cue. Inhibition of the PrL-PVT pathway selectively decreased drug-seeking behavior in STs, without affecting behavior in GTs, suggesting that the PrL-PVT acts to enhance the incentive motivational properties of a cocaine-cue selectively in STs. For the cocaine-primed reinstatement test, nose pokes into the active port did not result in delivery of cocaine or presentation of the cocaine-cue, however rats received an injection of cocaine prior to the session starting. Inhibition of the PrL-PVT pathway did not affect drug-seeking behavior during this test in either phenotype. Thus, the prelimbic cortical projections to the PVT act to mediate the

incentive motivational value of the cocaine-cue, but not the value of the cocaine itself, and does so selectively in STs.

In order to ensure that every rat received the same number of cocaine infusions and cocaine-cue presentations, infusion criterion were used. In agreement with previous findings, STs and GTs did not differ in behavior during cocaine self-administration (Saunders and Robinson 2010, Saunders and Robinson 2011, Saunders et al. 2013, Kuhn et al. 2018). A one-month abstinence period was used for this experiment, as previous work from our lab showed that a 2-week forced abstinence period was not sufficient to capture individual variation in cue-induced reinstatement of drug-seeking behavior (Kuhn et al. 2018). Work has shown that relative to a 2-wk forced abstinence period, a 1-mo period results in greater cue-induced drug-seeking behavior (Grimm et al. 2001). Rats also underwent the exact same number of extinction training sessions prior to the reinstatement tests to minimize the possibility of behavioral training affecting drug-seeking behavior during the test sessions. As previously found, STs and GTs did not differ in behavior during extinction training prior to the reinstatement tests (Yager and Robinson 2010, Saunders and Robinson 2011, Saunders et al. 2014, Kawa et al. 2016, Kuhn et al. 2018). This is pertinent, as PrL-PVT inhibition differentially affected drug-seeking behavior in STs and GTs during the test for cue-induced reinstatement but did not affect behavior during extinction training that followed. This suggests that inhibition of this pathway during cue-induced reinstatement does not affect subsequent behavior during extinction training. Relative to GTs, STs also showed greater cue-induced drug-seeking behavior as previously shown (Saunders and Robinson 2010, Saunders et al. 2013). However, inhibition of the PrL-PVT pathway selectively decreased cue-induced drug-seeking behavior in STs, such that they no longer differed from GTs. Thus, inhibiting this pathway eliminated individual differences in cue-

induced reinstatement. These results suggest that the PrL-PVT pathway acts to enhance the incentive motivational value of the cocaine-cue. Inhibiting this pathway then leads to a selective decrease in drug-seeking behavior in STs.

The results from this study are particularly intriguing, as they directly oppose our hypothesis that this pathway would act to inhibit the incentive motivational value of a cocaine-cue, not enhance it. Our hypothesis was based on recent findings in our lab showing that this pathway mediates sign-tracking and goal-tracking behavior such that it inhibits incentive salience attribution (Campus et al. 2018). However, while this work assessed the role of this pathway in Pavlovian learning (Campus et al. 2018), the current study utilized an instrumental procedure. Work has shown that the neural circuitry mediating these two forms of associative learning differ (Ostlund and Balleine 2007, Yin et al. 2008, Wassum et al. 2011, Gruart et al. 2015), and so it is possible that the role of the PrL-PVT pathway inherently differs between these two forms of learning. Additionally, previous work used an appetitive reward (e.g. food) while examining the role of this pathway in Pavlovian learning (Otis et al. 2017, Campus et al. 2018), but here we used cocaine as the reward. It is well known that drug-taking behavior results in neuroplastic changes within pathways in the motive circuit (for review see Kalivas and Volkow 2005, O'Brien 2009, Bobadilla et al. 2017, Dong et al. 2017). Thus, it is possible that the PrL-PVT pathway undergoes neuroplastic changes as a result of cocaine exposure, thereby changing its functional role. In fact, recent work has shown the cells in the PrL and posterior PVT are activated shortly after the conclusion of brief (2-weeks) cocaine self-administration training (Giannotti et al. 2018). It is believed that the PrL-PVT pathway becomes hyperactive after the cocaine-taking experience and contributes to relapse, as inhibiting this pathway after the last cocaine self-administration session resulted in a decrease in cue-induced drug-seeking behavior

(Giannotti et al. 2018). Thus, it is possible that cocaine experience may functionally alter the role of the PrL-PVT pathway in cue-reward learning. That is, whereas prior to cocaine experience this pathway acted to inhibit the expression of the incentive motivational properties of a reward-cue, following cocaine experience the PrL-PVT pathway instead enhances the incentive motivational value of the reward-cue. Interestingly, this function of the PrL-PVT pathway appears to be specific to STs, as cue-induced drug-seeking behavior was not affected by manipulation of this circuit in GTs. These findings therefore suggest that this pathway selectively mediates cue-induced drug-seeking behavior when the cocaine-cue has been attributed with an incentive motivational value (Saunders and Robinson 2010, Saunders et al. 2013), and not just a predictive value.

While the mechanisms by which inhibition of the PrL-PVT projection attenuates cue-induced drug-seeking behavior in STs is unknown, it is possible that inhibiting this pathway affects dopamine transmission within the nucleus accumbens (NAc). The PVT can elicit dopamine release within the NAc (Parsons et al. 2007), and dopamine signaling within this region contributes to the enhanced cue-induced drug-seeking behavior present in STs relative to GTs (Saunders et al. 2013). Here we show that CNO-induced c-fos activity differs between STs and GTs in the PVT. That is, inhibition of the PrL-PVT circuit appears to increase neuronal activity in the PVT in STs relative to GTs. Although we have yet to determine if these differences in neuronal activity are specific to cells expressing mCherry (i.e. with DREADDs), it is probable that such differences in PVT activity affect downstream neuromodulation in areas like the NAc. Ongoing studies are assessing CNO-induced c-fos activity in other brain regions, including the PrL and NAc, and will determine whether the differences we have revealed thus far in the PVT are specific to mCherry-expressing neurons.

All rats exhibited cocaine-primed reinstatement of drug-seeking behavior in this study. However, in contrast to previous findings (Saunders and Robinson 2011), STs and GTs did not differ in drug-seeking behavior during this reinstatement test. The dose given in this experiment (15 mg/kg, i.p.) was the same previously given that resulted in differences in drug-seeking behavior between STs and GTs (Saunders and Robinson 2011). However, several methodological differences exist between the current study and that done by Saunders and Robinson (2011). In the current study, rats underwent one-month of forced abstinence, extinction training and cue-induced reinstatement, as well as another period of extinction training and a brief abstinence period prior to undergoing cocaine-induced reinstatement. Thus, rats did not receive any cocaine for approximately 2-months leading up to the cocaine-primed reinstatement test. In previous work showing individual differences in cocaine-primed reinstatement, rats did not undergo a forced abstinence period, and underwent the reinstatement test approximately one week following cocaine experience (Saunders and Robinson 2011). It is possible that the presence of the forced abstinence period in our study, which is known to result in cellular adaptations following cocaine experience (for review see Grimm et al. 2003, Robinson et al. 2004), resulted in the differences observed between our results and previous findings. The time between cocaine self-administration training and receiving the cocaine prime, as well as the behavioral training that occurred during this time, greatly differed between the two studies as well. These factors may all have contributed to our lack of seeing individual variation in cocaine-primed reinstatement in this study. Furthermore, rats exhibited stereotyped behaviors during the reinstatement test, and although there were no significant differences between phenotypes or treatment in these behaviors, it remains a possibility that cocaine-induced stereotypy affected the outcome measures of drug-seeking behavior we observed. Nonetheless, it appears that STs and

GTs may only differ in cocaine-primed reinstatement of drug-seeking behavior when the time between cocaine-experience and reinstatement are short (Saunders and Robinson 2011). From the current findings, however, we conclude that inhibition of the PrL-PVT pathway does not affect cocaine-induced drug-seeking behavior in either phenotype, suggesting that this pathway specifically mediates the cue-reward relationship, and not the motivation for the drug itself.

The results of this study highlight the complex role of the cortical projections from the PrL to the PVT in cue-reward learning. In Pavlovian learning, this pathway appears to inhibit the expression of incentive salience toward a food-cue (Campus et al. 2018). However, we report here that PrL-PVT inhibition selectively attenuates cue-induced drug-seeking behavior in STs, and does not affect cocaine-primed reinstatement of drug-seeking behavior in either phenotype. These data suggest that the projections from the PrL to the PVT act to enhance the incentive motivational value of a cocaine-cue selectively in rats with a high relapse propensity (STs), but does not mediate the motivational value of the cocaine itself. Future studies should further explore the mechanisms by which the PrL-PVT pathway mediates individual variation in cue-induced drug-seeking behavior.

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Behavior	Definition
Stereotyped behaviors	
Circling (i.e. “spinning”)	Four consecutive 90° turns occurring within close succession of one another
Head movements	Lateral head movements made when the back paws remain on the flooring and in the same position for a minimum of 2-sec
Non-stereotyped behaviors	
Active port orientation	Oriented toward the active port, but not advancing toward the port
Active port approach	Approached the active port, but made no pokes into the port
Immobility	Time during which all 4 paws remain on the flooring in the same position for a minimum of 2-sec, which no other behaviors co-occurring

Table 3.1 Table of behaviors quantified by a blind observer during video analysis of the test for cocaine-primed reinstatement. Behaviors were scored in 30-sec bins every 10-min for the first 60-min of the session. Time spent doing each behavior was recorded per bin.

	Stereotyped behavior			Non-stereotyped behavior		
	Circling	Head movement: no port entry	Head movement: port entry	Immobility	Active port orientation: no movement	Active port approach: no entry
ST VEH (n=4)	0.9% +/- 0.9%	9.9% +/- 4.1%	0.0% +/- 0.0%	6.4% +/- 4.7%	0.0% +/- 0.0%	10.6% +/- 7.9%
ST CNO (n=3)	0.3% +/- 0.3%	0.0% +/- 0.0%	6.0% +/- 3.9%	9.1% +/- 4.6%	7.3% +/- 7.3%	10.7% +/- 3.0%
GT VEH (n=10)	2.0% +/- 0.9%	1.6% +/- 1.1%	7.6% +/- 6.1%	7.4% +/- 4.2%	0.2% +/- 0.2%	16.0% +/- 2.7%
GT CNO (n=9)	2.7% +/- 1.2%	2.7% +/- 1.4%	0.0% +/- 0.0%	2.3% +/- 1.2%	3.4% +/- 2.3%	15.2% +/- 5.6%

Table 3.2 Percent of time exhibiting stereotyped and non-stereotyped behavior during cocaine-primed reinstatement. Data are represented as mean percent time +/- SEM. Rats exhibited minimal stereotyped behavior during cocaine-primed reinstatement.

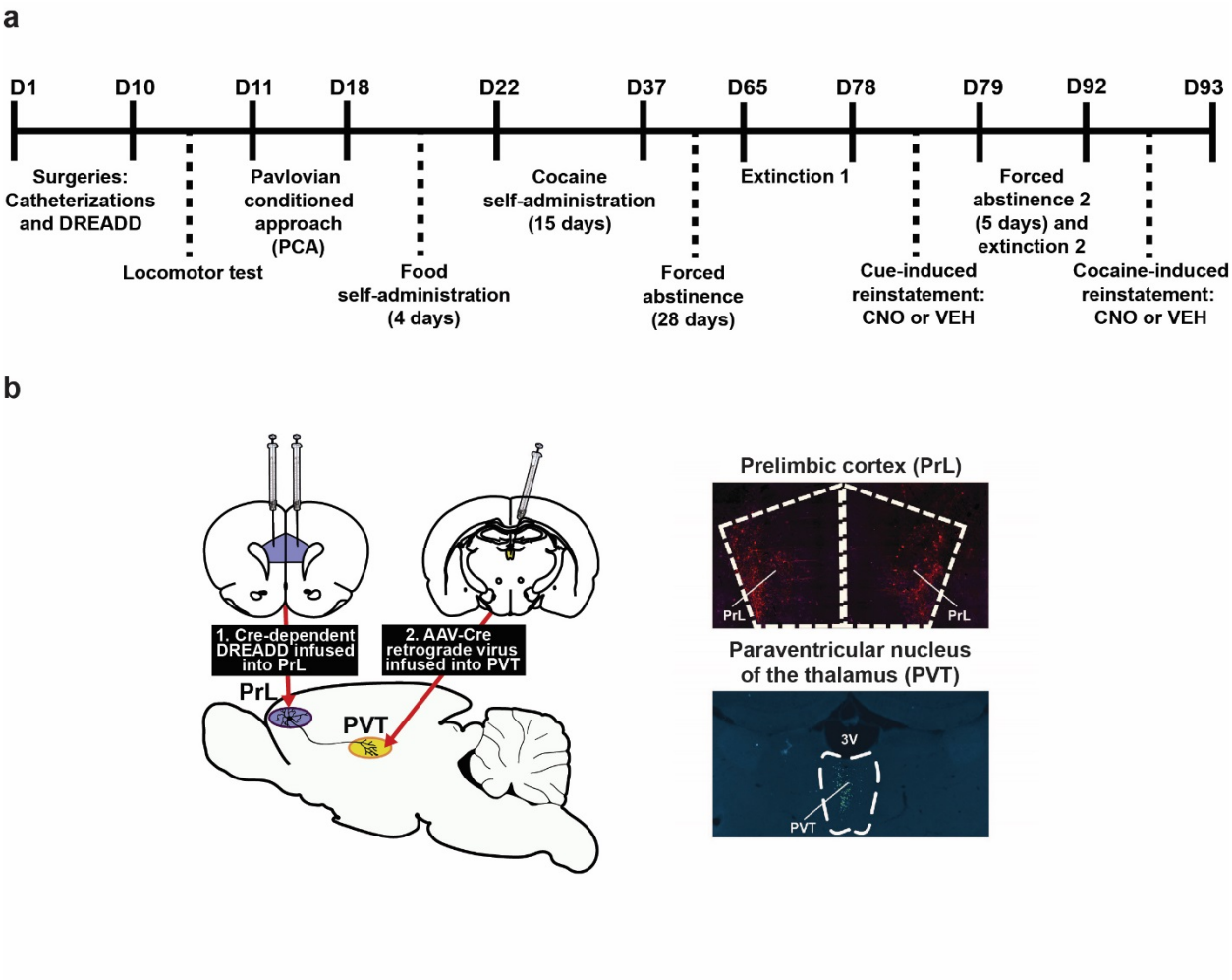

Fig. 3.1 Experimental timeline. **a)** Rats underwent surgeries for indwelling jugular catheter implantation and viral infusions into the PrL, aPVT and pPVT for G_i DREADD expression in the PrL-PVT pathway. Rats were then characterized based on their locomotor response to a novel environment and PCA training. Food self-administration (4 days) and cocaine self-administration (15 days) followed, with a subsequent 28-day forced abstinence period. Daily extinction sessions occurred for 13 consecutive days prior to the cue-induced reinstatement test. Rats were given an injection (i.p.) of either vehicle (VEH) or 5 mg/kg clozapine-N-oxide (CNO) to activate the G_i DREADD prior to the reinstatement test. Following cue-induced reinstatement, rats underwent 5 days of forced abstinence then daily extinction training for 8 consecutive days before cocaine-induced reinstatement. Prior to the reinstatement test, rats were given the same treatment (vehicle of CNO) as the cue-induced reinstatement, as well as a 15 mg/kg injection of cocaine immediately before being placed into the testing chamber. **b)** Schematic illustrating DREADD surgery and fluorescent images of DREADD expression within the PrL, and AAV-Cre expression in the PVT. During surgery, the Cre-dependent DREADD is infused bilaterally into the PrL and the AAV-Cre is infused into the PVT. The presence of Cre results in the DREADD construct being flipped, allowing for transcription and translation into a functional receptor selectively within the PrL-PVT pathway.

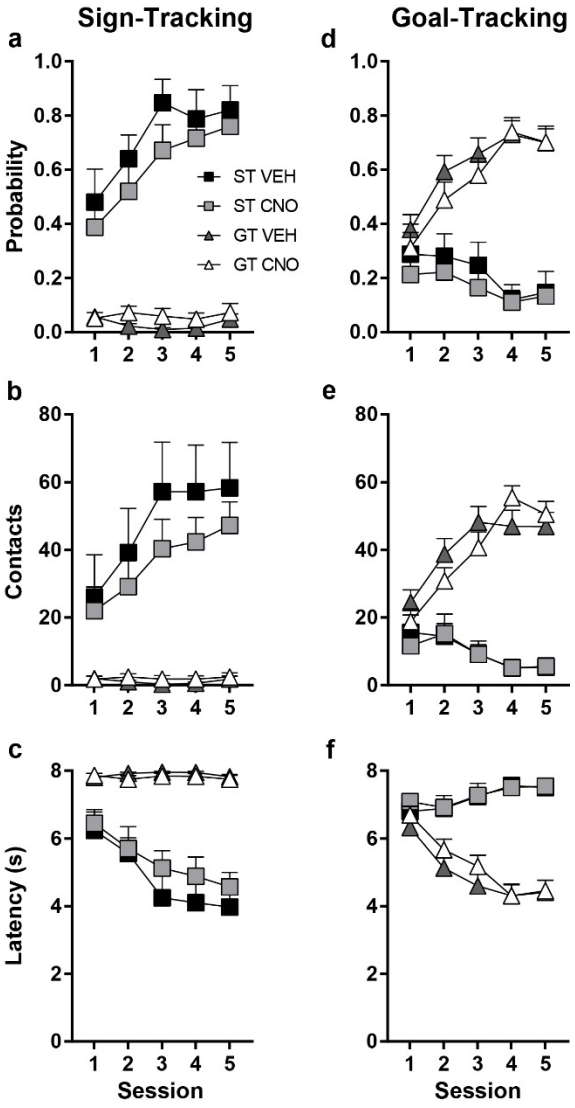

Fig. 3.2 Individual differences in Pavlovian conditioned approach (PCA) training. Mean + SEM for **a)** the probability to approach the lever during its presentation, **b)** the number of lever contacts made, **c)** latency to approach the lever, **d)** probability to contact the food magazine during lever-CS presentation, **e)** number of food magazine contacts, and **f)** latency to contact the food magazine during lever-CS presentation. There was a significant Effect of Phenotype, Session, and Phenotype x Session interaction for all measures ($p<0.01$). Sign-trackers (VEH, n=6; CNO, n=9) acquired lever-CS directed behavior, and goal-trackers (VEH, n=15; CNO, n=15) acquired food-magazine directed behavior. Rats did not receive any treatment prior to PCA training but data are illustrated according to the treatment (i.e. VEH or CNO) rats will receive prior to the reinstatement tests.

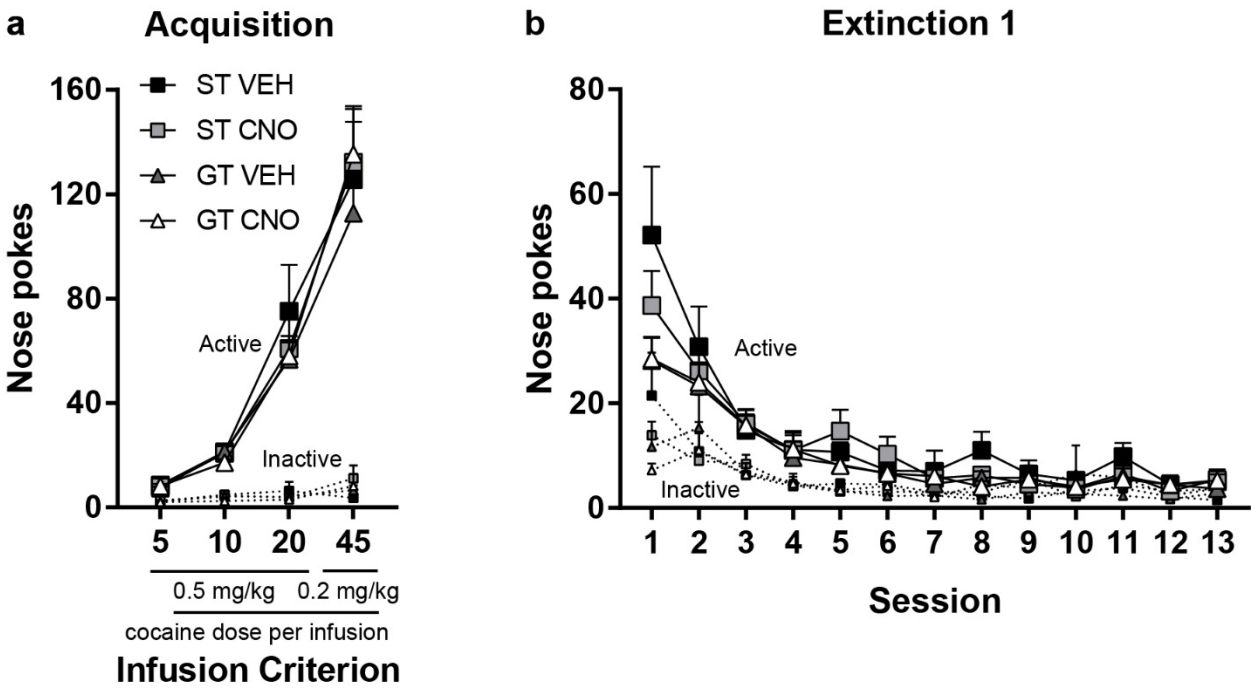

Fig. 3.3 Acquisition of cocaine self-administration and extinction training. **a)** Mean + SEM for nose pokes into the inactive and active port for STs and GTs during acquisition of cocaine self-administration across infusion criterion (IC). Rats made more pokes into the active port ($p<0.001$) across infusion criterion ($p<0.001$), and behavior did not differ based on assigned treatment group (VEH or CNO) or phenotype (ST or GT). Cocaine infusions at IC5, IC10 and IC20 were at a dose of 0.5 mg/kg/infusion, and decreased to 0.2 mg/kg/infusion for IC45. **b)** Mean + SEM for nose pokes made into the inactive and active ports across 13 sessions of extinction training in STs and GTs. Rats decreased drug-seeking behavior across training sessions ($p<0.0001$), and behavior did not differ based on assigned treatment group (VEH or CNO) or phenotype (ST or GT). (ST VEH, n=6, ST CNO, n=9, GT VEH, n=16, GT CNO, n=15)

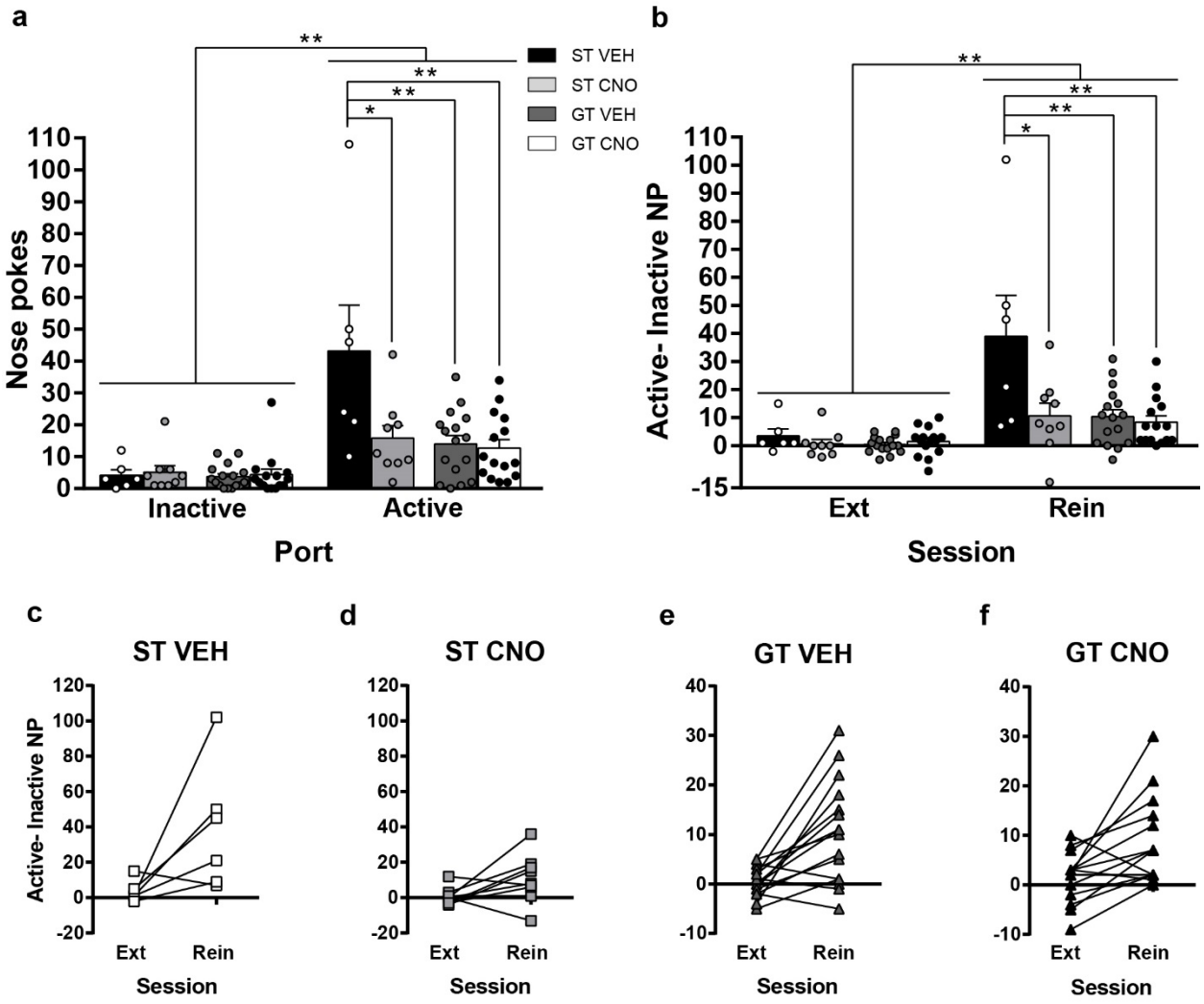

Fig. 3.4 Effects of chemogenetic inhibition of the PrL-PVT pathway on cue-induced reinstatement of drug-seeking behavior. **a)** Mean + SEM for nose pokes into the inactive and active port during the test for cue-induced reinstatement. There was a significant Effect of Port ($p<0.0001$), Phenotype ($p=0.002$) and Treatment ($p=0.01$). All interactions were significant, including the Port x Phenotype x Treatment interaction ($p=0.02$). The ST VEH group made more pokes into the active port compared to the GT VEH ($p< 0.01$) and GT CNO ($p= 0.001$) groups. Inhibition of the PrL-PVT pathway selectively decreased drug-seeking behavior in STs ($p<0.05$) compared to the ST VEH group. **b)** Mean + SEM for active-inactive nose pokes (NP) made during the last extinction training session (Ext) and the test for cue-induced reinstatement (Rein). All rats showed reinstatement of drug-seeking behavior (Effect of Session, $p<0.0001$), and inhibition of the PrL-PVT pathway selectively decreased overall drug-seeking behavior in STs ($p<0.05$) compared to rats in the ST VEH group. Active-inactive nose pokes (NP) made during the last extinction training session (Ext) and the test for reinstatement (Rein) for each rat in the **c)** ST VEH, **d)** ST CNO, **e)** GT VEH, and **f)** GT CNO groups. (ST VEH, n=6, ST CNO, n=9, GT VEH, n=16, GT CNO, n=15) *$p<0.05$, **$p<0.01$

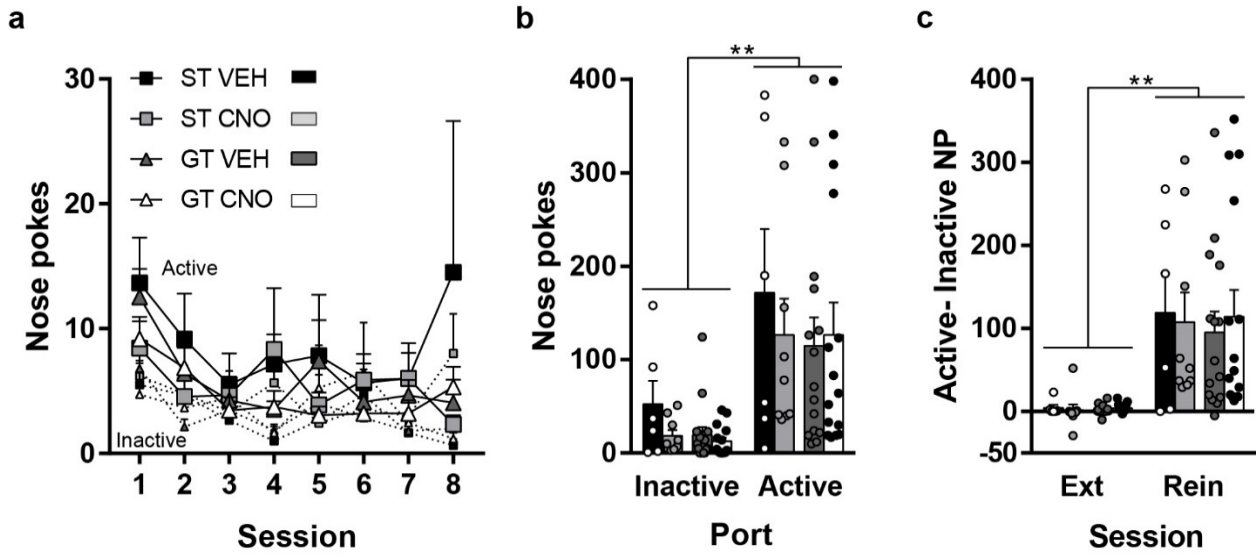

Fig. 3.5 Extinction 2 training and cocaine-primed reinstatement of drug-seeking behavior. **a)** Mean + SEM for nose pokes made into the inactive and active port across 8 sessions of extinction training in STs. All rats decreased drug-seeking behavior across training sessions ($p<0.0001$). Extinction rate did not differ between phenotype (ST or GT) or treatment (VEH or CNO). **b)** Mean + SEM for nose pokes into the inactive and active port during a test for cocaine-induced reinstatement. All rats made more pokes into the active port compared to the inactive port (Effect of Port, $p=0.001$). **c)** Mean + SEM for active-inactive nose pokes (NP) during the last extinction training session (Ext) and the test for cocaine-induced reinstatement (Rein). All rats showed reinstatement of drug-seeking behavior (Effect of Session, $p<0.0001$). (ST VEH, n=6, ST CNO, n=9, GT VEH, n=15, GT CNO, n=15) **$p<0.01$

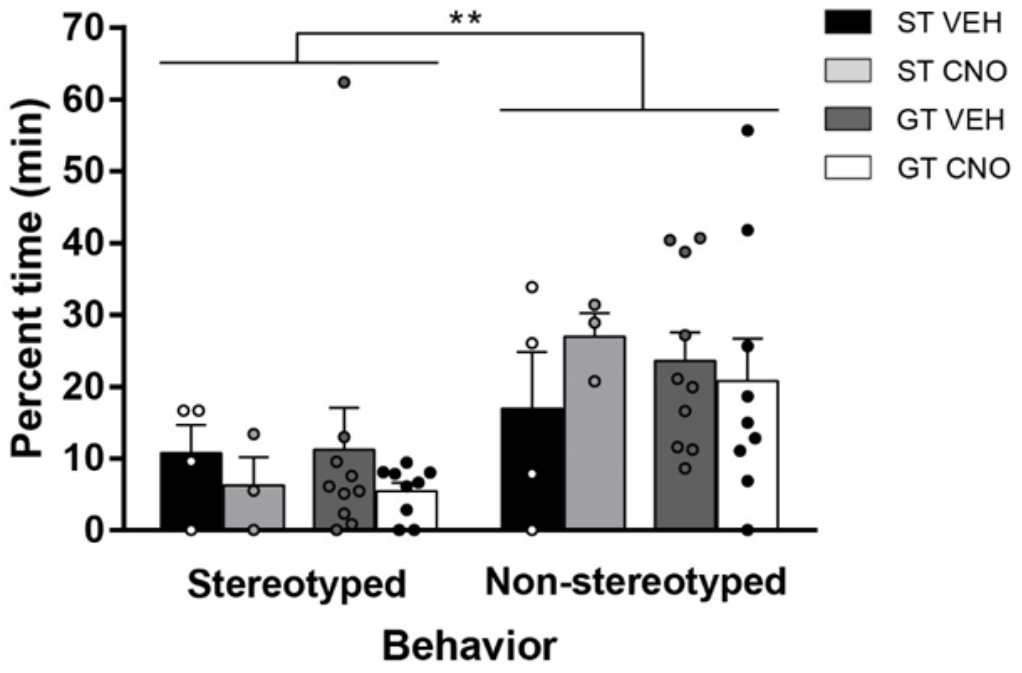

Fig. 3.6 Stereotyped behavior and non-stereotyped behavior during cocaine-primed reinstatement. Mean + SEM for percent time exhibiting stereotyped behavior during the first hour of the test for cocaine-primed reinstatement. All rats exhibited more non-stereotyped than stereotyped behavior ($p<0.001$), and behavior did not differ based on phenotype ($p=0.99$) or treatment ($p=0.84$). (ST VEH, n=4; ST CNO, n=3; GT VEH, n=10; GT CNO, n=9) **$p<0.01$

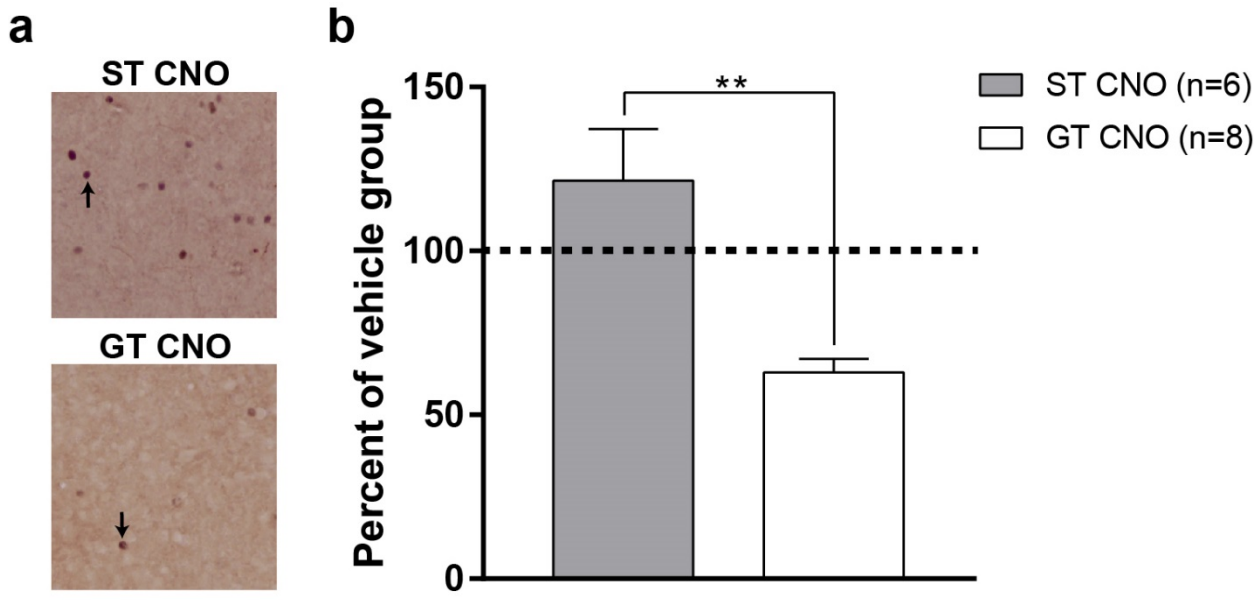

Fig. 3.7 Percent PVT c-fos density in CNO-treated rats relative to vehicle-treated rats. **a)** Representative images of c-fos expression in the PVT of a ST and GT following PrL-PVT inhibition (i.e. CNO administration). Black arrows indicate c-fos protein. **b)** Mean + SEM for percent c-fos density throughout the entire PVT (anterior, middle and posterior) relative to the vehicle-treated group (i.e. baseline). Inhibition of the PrL-PVT pathway results in a decrease in c-fos density in GTs relative to vehicle-treated rats ($p<0.001$), and no effect in STs relative to vehicle-treated rats ($p=0.23$). Inhibition of the PrL-PVT pathway differentially affected PVT c-fos density (% vehicle) STs relative to GTs ($p=0.002$; $^{**}p<0.01$). (VEH group, n=13 (ST, n=3; GT, n=10); ST CNO, n=6; GT CNO, n=8)

Appendix A

Abstract

Data from the rats in Chapter 3 were also analyzed within the context of the high-responder (HR)/ low-responder (LR) model. This model captures individual variation in the acquisition of drug self-administration based on locomotor response to an inescapable novel environment. However, outbred HRs and LRs do not differ in other addiction-related behaviors including, cue-induced and cocaine-primed reinstatement of drug-seeking behavior. The aim of this study was to assess whether inhibition of the PrL-PVT circuit affected cue- or cocaine-induced drug-seeking behavior in two different models of individual variation in addiction vulnerability. To test this, rats were classified as HRs and LRs, based on their locomotor response to novelty, before undergoing self-administration, extinction, and reinstatement procedures as described above (for Chapter 3). We found that HRs show greater cue-induced drug-seeking behavior compared to LRs. However, PrL-PVT inhibition did not affect drug-seeking behavior in either the cue- or cocaine-induced reinstatement test. These findings reinforce the notion that this pathway selectively mediates individual differences in the propensity to attribute incentive salience to a cocaine cue, as captured by the ST/GT animal model.

Introduction

One of the first animal models used to assess individual variation in addiction-related behaviors was the high-responder (HR)/ low-responder (LR) model (Piazza et al. 1989, Piazza et

al. 1998). In this model, rats are placed in an inescapable novel environment and are then separated into HRs and LRs based on their level of locomotor activity; with HRs exhibiting greater levels of novelty-induced locomotion. This model has specifically been associated with individual variation in the acquisition of drug self-administration, as HRs self-administer cocaine (Piazza et al. 2000, Mantsch et al. 2001, Ferris et al. 2013), amphetamine (Piazza et al. 1989, Piazza et al. 1990, Piazza et al. 1991, Piazza et al. 1998, Klebaur et al. 2001, Cain et al. 2008), nicotine (Suto et al. 2001), and heroin (Lamarque et al. 2001) at a faster rate compared to LRs. After prolonged cocaine self-administration, however, outbred animals characterized as HRs and LRs do not differ in other-addiction related behaviors (Deroche-Gamonet et al. 2004). This includes the motivation to work for the drug, compulsive drug-seeking behavior when the drug is not available, and working for the drug in the face of adverse consequences (Deroche-Gamonet et al. 2004). Drug-seeking behavior during tests for cocaine-primed and cue-induced reinstatement also do not differ between the two phenotypes after prolonged cocaine self-administration (Deroche-Gamonet et al. 2004). Thus, this outbred animal model appears to solely capture individual variation in the initiation of drug-taking behavior.

Given that locomotor response to novelty has been associated with individual differences in the acquisition of drug-taking behavior, but not differences in relapse propensity; it is, perhaps, not too surprising that this “sensation-seeking” trait and the propensity to attribute incentive salience to rewards cues appear to be unrelated in outbred rat populations (Robinson and Flagel 2009). In the current study, however, we characterized rats according to both traits, to assess whether inhibition of the PrL-PVT circuit would differentially affect drug-seeking behavior in each of the phenotypes (HR, LR, ST, GT). Rats underwent a test for locomotor response to a novel environment and were classified as HRs and LRs using a median split for

cumulative locomotor movements. Rats then underwent Pavlovian conditioned approach training followed by cocaine self-administration training, extinction and a test for cue-induced and cocaine-primed reinstatement as described in Chapter 3. Briefly, prior to the test for cue-induced reinstatement, rats received an injection of a vehicle solution or clozapine-N-oxide (CNO) to chemogenetically inhibit the projections from the prelimbic cortex (PrL) to the paraventricular nucleus of the thalamus (PVT). Rats then underwent additional extinction training and a test for cocaine-primed reinstatement. Prior to the test, rats received an injection of that (vehicle or CNO) previously given. The objective of this study was to assess whether PrL-PVT inhibition similarly affects cocaine-primed and cue-induced drug-seeking behavior in rats using two different models of addiction vulnerability: one that captures individual variation in the acquisition of cocaine self-administration (HR/LR model; Appendix); and one that captures individual variation in reinstatement propensity (ST/ GT model; Chapter 3).

Methods

Subjects

All data in this Appendix are from the same rats (i.e. rats classified as STs and GTs) included in Chapter 3. However, data will be analyzed and discussed within the context of the HR/LR model, not the ST/GT model. Male heterogeneous stock (HS) rats from a breeding colony at the Medical College of Wisconsin were used for this study. Rats had ad libitum access to food and water throughout the study and were single-housed after surgeries in a climate-controlled room with a 12-hour light: dark cycle (lights came on at either 06:00 h or 07:00 h depending upon daylight savings time). All training occurred between 08:00 h and 18:00 h (light

cycle), with specific testing times as indicated in Chapter 3. Supporting Figure S3.1 shows the experimental timeline.

Surgical procedures

All rats underwent surgery for indwelling jugular catheter implantation and viral infusions for inhibitory (G_i) DREADD (Designer Receptors Exclusively Activated by Designer Drugs) expression in the PrL-PVT pathway, as described in Chapter 3. Rats were given a minimum of five days to recover from surgery before behavioral training started.

Locomotor test

After the surgical recovery period, rats underwent a 60-min locomotor test. Testing chambers (43 x 21.5 cm floor area, 25.5 cm high) were outfitted with infrared beams to track both lateral (beams 2.3 cm above grid floor) and rearing (beams 6.5 cm from grid floor) movements. All testing occurred between 10:00 h and 16:00 h under red light. Lateral and rearing movements were recorded in 5-min increments. At the conclusion of the test, rats were returned to their home cages in the colony room. Cumulative locomotor movements (i.e. rearing and lateral movements) were used to classify rats into their respective phenotypes. Rats for this study were run in six separate cohorts and classified as HRs and LRs within each cohort using a median split based on the cumulative locomotor movements. Following locomotor testing, rats underwent Pavlovian conditioned approach (PCA) training as described in Chapter 3.

Self-administration training, extinction training, and reinstatement tests

All training and testing procedures are the same as previously described in Chapter 3.

Video analysis for cocaine-induced stereotyped behaviors

Stereotyped (i.e. circling, head movements but no port entry, head movements and port entry) and non-stereotyped behavior (immobility, orientation toward the active port without movement toward it, approach to the active port without port entry) was quantified as previously described in Chapter 3. A more detailed description of the criteria for each behavior can be found in Supporting Table 3.1. Behavior was analyzed for 30-sec every ten minutes for the first hour of the reinstatement test, as this is when peak drug-seeking behavior occurred. The percentage of time (min) rats spent exhibiting each behavior across the six bins was quantified.

Statistical analyses

The effects of Phenotype (HR, LR), Treatment (VEH, CNO), and Port (active, inactive) were assessed on the number of nose pokes made during cocaine self-administration (IC: 5, 10, 20, 45) and extinction training (Sessions: 1-13) using a linear mixed-effects model. To assess individual variation in the acquisition of cocaine self-administration, the average interinfusion interval for each IC was analyzed with Phenotype, Treatment and IC as the independent variables. For the interinfusion interval analyses, outliers were identified using the Grubb's outlier test (alpha=0.05). Data are reported both with, and without, the outliers included in analyses. Interinfusion intervals were assess using Phenotype, Treatment and IC as the independent variables. All linear mixed-effects model analyses were conducted using SPSS Statistics Program (Statistical Package for the Social Sciences), version 22 (IBM, Armok, NY). For each analysis, the best covariance structure was chosen based on the lowest Akaike's information criterion. The relationship between the rate of extinction and drug-seeking behavior

during the subsequent reinstatement test was also assessed using SPSS. To do so, a quadratic regression model was fit to the extinction training curve for each rat, and then the quadratic term was regressed onto the number of nose pokes made into the active port during the reinstatement test.

Data for the locomotor test was analyzed using a three-way ANOVA, with Phenotype, Treatment and Cohort (1-6) as the independent variables. Average session length for cocaine self-administration training was analyzed using a three-way ANOVA, Phenotype, Treatment and IC as the independent variables. Nose pokes made during the reinstatement tests were analyzed using a three-way ANOVA with Phenotype, Treatment and Port (active or inactive) as the independent variables. A repeated-measures ANOVA was used to assess differences in drug-seeking behavior between the reinstatement tests and last extinction training session with Phenotype, Treatment and Session (Extinction or Reinstatement) as the independent variables, and the number of responses in the "active-inactive" ports as the dependent variable. A repeated measures ANOVA was used to assess the effect of Phenotype and Treatment on the percent of time engaged in stereotyped behavior versus non-stereotyped behavior (Category) during the cocaine-primed reinstatement test. Nose pokes made during the last extinction training sessions (session 13) were analyzed using a three-way ANOVA, with Phenotype, Treatment and Port as the independent variables. Correlational analyses between PCA index and locomotor activity score were also performed. All ANOVAs and correlational analyses occurred using StatView, version 5.0 (SAS Institute Inc., Cary, NC, USA). Correlations between nose pokes made into the active port during the test for reinstatement and other behavioral measures throughout training were also conducted using StatView.

Post-hoc analyses for significant interactions were analyzed using Bonferroni tests to correct for multiple comparisons. For all tests, statistical significance was set at $p<0.05$. Outliers for the cocaine-primed reinstatement test and video analysis were identified using the Grubb's outlier test (alpha=0.05).

Results

HRs show greater locomotor activity in a novel environment compared to LRs

HRs showed greater locomotor activity (i.e. rearing and lateral movements) in response to a novel environment compared to LRs (Effect of Phenotype, $F_{1,42}=63.02$, $p<0.0001$) (Supporting Figure S3.2). Rats did not differ in locomotor response to novelty based on the treatment they were assigned to subsequently receive on the test day (Effect of Treatment, $F_{1,42}=0.04$, $p=0.85$). Additionally, locomotor activity of rats did not differ between cohorts (Effect of Cohort, $F_{1,38}=1.08$, $p=0.31$).

Locomotor activity does not correlate with PCA index

Correlations between cumulative locomotor activity and PCA index were performed separately for STs and GTs. In agreement with prior reports using outbred populations (Robinson and Flagel 2009), there was not a significant correlation between these two measures in STs ($r^2=0.10$, $p=0.25$) or GTs ($r^2=0.01$, $p=0.69$), indicating that these two traits – "sensation-seeking" and the propensity to attribute incentive salience to reward cues – are independent from one another.

Cocaine self-administration

All rats discriminated between the active and inactive port throughout self-administration training (Effect of Port, $F_{1,85}$=251.14, p<0.0001), and increased drug-taking behavior as training progressed (Effect of IC, $F_{3,85}$=103.23, p<0.0001) (Supporting Figure S3.3a). There was a significant interaction between IC and Port ($F_{3,85}$=76.64, p<0.0001), revealing that the increase in responding with each successive IC was specific to the active port (p<0.0001). There was also a significant interaction between Phenotype and IC ($F_{3,85}$=4.07, p=0.01), with HRs showing greater drug-taking behavior during IC10 (p=0.01) and IC20 (p=0.02) compared to LRs (Supporting Figure S3.3a). In addition, there was a significant Treatment by IC interaction ($F_{3,85}$=3.35, p=0.02), and post-hoc analyses showed that rats in the vehicle treatment group showed greater drug-taking behavior at IC10 (p=0.04) than rats in the CNO treatment group (Supporting Figure S3.3a). However, the two treatment groups did not differ at IC5 (p=0.67), IC20 (p=0.32) or IC45 (p=0.26).

To assess individual variation in the acquisition of cocaine self-administration, the interinfusion interval (III), or time (min) between pokes into the active port that resulted in an infusion of cocaine, was analyzed for each IC. Rats decreased their III as training progressed (Effect of IC, $F_{3,84}$=18.24, p<0.0001), but HRs showed lower III across training compared to LRs (Effect of Phenotype, $F_{1,54}$=8.38, p=0.01) (data not shown in graphs). Statistical outliers were identified using the Grubb's outlier test, and once removed (IC20, LR CNO: 1; IC45, LR CNO: 1) data was reanalyzed. For visualization purposes, data is shown with outliers removed (Supporting Figure S3.3b). After removing outliers, there were still significant Effects of Phenotype ($F_{1,50}$=6.78, p=0.01) and IC ($F_{3,74}$=30.30, p<0.0001). However, a significant Phenotype by IC interaction ($F_{3,74}$=4.16, p=0.01) emerged. Post-hoc analyses showed that HRs had lower III compared to LRs during IC5 (p=0.01), but did not differ during IC10 (p=0.06),

IC20 (p=0.07) and IC45 (p=0.31) (Supporting Figure S3.3b). Session length was also analyzed across cocaine self-administration training. Session length increased across IC (Effect of IC, $F_{3,80}$=5.14, p=0.003) as would be expected, however HRs finished sessions faster than LRs across training (Effect of Phenotype, $F_{1,83}$=14.82, p<0.0001). This is to be expected as HRs showed shorter III than LRs. Thus, despite the fact that this paradigm controlled for the maximum number of infusions received, we were able to capture individual differences in the acquisition of drug-taking behavior in outbred HR/LR rats, as other studies have reported (Piazza et al. 1989, Piazza et al. 1990, Piazza et al. 1998, Piazza et al. 2000, Klebaur et al. 2001, Mantsch et al. 2001, Ferris et al. 2013).

Extinction 1 training

All rats decreased nose pokes into the active and inactive port as extinction training progressed (Effect of Session, $F_{12,84}$=26.67, p<0.001). Rats also differentiated between the active and inactive port (Effect of Port, $F_{1,84}$=41.94, p<0.0001) (Supporting Figure S3.4), but there was a significant Session by Port interaction ($F_{12,84}$=6.19, p<0.0001) and rats did not discriminate between the active and inactive port on session 9 (p=0.12), 10 (p=0.93) or 11 (p=0.07). There was also a significant Phenotype x Treatment interaction ($F_{1,84}$=11.54, p<0.001), suggesting that rats differed in behavior during extinction training based on test day treatment. Post-hoc analyses showed that rats in the vehicle treatment group differed in nose pokes made into the active and inactive port across training compared to rats in the CNO treatment group (p=0.001) (Supporting Figure S3.4). Additionally, test day treatment affected pokes into the active and inactive port within each phenotype (HR, p=0.01; LR, p=0.04) (Supporting Figure S3.4). Despite differences in the rate of extinction between phenotypes and treatment groups, there was no Effect of

Phenotype ($F_{1,84}$=2.20, p=0.14) or Treatment ($F_{1,84}$=1.97, p=0.16) on nose pokes made into the active and inactive port during the last extinction training session. Thus, by the conclusion of extinction training, all rats were behaving the same.

PrL-PVT inhibition does not affect cue-induced drug-seeking behavior, however HRs show greater drug-seeking behavior compared to LRs

There was a significant Effect of Phenotype ($F_{1,84}$=5.13, p=0.03) and Treatment ($F_{1,84}$=4.16, p=0.04) and all rats differentiated between the two ports (Effect of Port, $F_{1,84}$=32.78, p<0.0001), with HRs and LRs both making more pokes into the active port compared to the inactive port (p<0.001 for both) (Supporting Figure S3.5a). A significant Port x Phenotype interaction ($F_{1,84}$=6.92, p=0.01) showed that HRs made more pokes into the active port compared to LRs (p<0.05), but the two phenotypes did not differ in pokes made into the inactive port (p=0.48), suggesting that HRs shows greater cue-induced drug-seeking behavior compared to LRs (Supporting Figure S3.5a). Post-hoc analyses from a significant Phenotype by Treatment interaction ($F_{1,84}$=6.35, p=0.01) revealed that HRs in the vehicle group made more nose pokes into both ports compared to LRs treated with vehicle (p=0.03), suggesting that HRs show greater responding in general. There was not a significant Phenotype x Treatment x Port interaction ($F_{1,84}$=1.15, p=0.29).

To compare drug-seeking behavior between the last extinction training session and the cue-induced reinstatement test, “active-inactive” nose pokes were assessed between the two sessions, as described in Chapter 3. Using this metric, all rats showed greater drug-seeking behavior during the cue-induced reinstatement test compared to the last extinction training session (Effect of Session, $F_{1,42}$=28.01, p<0.0001) (Supporting Figure S3.5b). There was a

significant Effect of Phenotype ($F_{1,42}$=8.39, p=0.01) and Treatment ($F_{1,42}$=4.92, p=0.03). Significant interactions between Session and Phenotype ($F_{1,42}$=5.62, p=0.02) and Session and Treatment ($F_{1,42}$=4.79, p=0.03) also existed. That is, while there were not significant differences between Phenotypes (p=0.26) or Treatment (p=0.96) during the last extinction session, HRs showed greater drug-seeking behavior compared to LRs during the reinstatement test (p=0.03) regardless of treatment given (p=0.10) (Supporting Figure S3.5b). These data suggest that HRs and LRs shows individual variation in cue-induced reinstatement of drug-seeking behavior, but the PrL-PVT pathway does not mediate this difference.

Interestingly, there was a significant correlation between nose pokes made into the active port during the test for cue-induced reinstatement and cumulative locomotor score for rats in the HR VEH group (r^2=0.71, p=0.01), but not rats in the other groups (HR CNO, r^2=0.05, p= 0.49; LR VEH, r^2=0.001, p=0.92; LR CNO, r^2=0.13, p= 0.26). This suggests that greater locomotor activity in a novel, inescapable environment can predict levels of cue-induced reinstatement of drug-seeking behavior selectively in HR rats, and inhibition of the PrL-PVT pathway disrupts this relationship. The PrL-PVT pathway may, therefore, contribute to cue-induced relapse propensity in HRs, even though direct manipulation of this pathway did not affect behavior in HRs during the reinstatement test.

Rats do not differ in extinction training after cue-induced reinstatement

All rats decreased nose pokes made into the active and inactive port across extinction training sessions (Effect of Session, $F_{7,84}$=9.73, p<0.0001) regardless of phenotype (Effect of Phenotype, $F_{1,84}$=1.35, p=0.25) or test day treatment (Effect of Treatment, $F_{1,84}$=0.45, p=0.50) (Supporting Figure S3.6a). Rats differentiated between the active and inactive port (Effect of

Port, $F_{1,84}$=7.05, p=0.01), but did so less as training progressed (Session x Port interaction, $F_{7,84}$=2.56, p=0.02) (Supporting Figure S3.6a). Post-hoc analyses revealed that rats did not differentiate between the two ports during session 3 (p=0.84), 5 (p=0.59), 6 (p=0.92) and 8 (p=0.07).

PrL-PVT inhibition does not affect cocaine-primed drug-seeking behavior, and HRs and LRs do not differ in drug-seeking behavior

Rats received a 15 mg/kg injection (i.p.) of cocaine prior to the start of the cocaine-primed reinstatement test. As mentioned in Chapter 3, one rat (LR VEH) was identified as an outlier and excluded from statistical analyses due to cocaine-induced stereotyped behavior resulting in an excessive number of pokes into the active port (~4500 pokes). All rats made more pokes into the active port than the inactive port (Effect of Port, $F_{1,82}$=28.30, p<0.0001) (Supporting Figure S3.6). HRs and LRs did not differ in behavior during the reinstatement test (Effect of Phenotype, $F_{1,82}$=0.32, p=0.57). Inhibition of the PrL-PVT pathway also does not appear to mediate drug-seeking behavior in either HRs or LRs during cocaine-primed reinstatement (Effect of Treatment, $F_{1,82}$=0.48, p=0.49) (Supporting Figure S3.6). Drug-seeking behavior (active-inactive nose pokes) between the last extinction training session and reinstatement test was also analyzed. All rats showed more drug-seeking behavior during the reinstatement test relative to the extinction training session (Effect of Session, $F_{1,41}$=41.05, p<0.0001). Behavior did not differ in either session based on phenotype (Effect of Phenotype, $F_{1,41}$=0.04, p=0.84) or treatment group (Effect of Treatment, $F_{1,41}$=0.04, p=0.84), and there were not significant interactions.

In an effort to account for variance in drug-seeking behavior observed during this test, correlations between nose pokes into the active port and other behavioral measures during training were assessed. There were no significant correlations between drug-seeking behavior during cue-induced and cocaine-primed reinstatement (HR VEH, r^2=0.08, p= 0.50; HR CNO, r^2=0.02, p= 0.68; LR VEH, r^2=0.24, p= 0.09; LR CNO, r^2<0.001, p= 0.97). Cumulative locomotor movements made during the locomotor test also did not correlate with drug-seeking behavior during the test for cocaine-primed reinstatement (HR VEH, r^2=0.01, p= 0.83; HR CNO, r^2=0.09, p= 0.34; LR VEH, r^2=0.18, p= 0.15; LR CNO, r^2=0.004, p= 0.85).

Cocaine-induced stereotyped behaviors during cocaine primed-reinstatement

Stereotyped behaviors during cocaine-primed reinstatement were assessed in a subset of rats (HR VEH, n=5; HR CNO, n=8; LR VEH, n=9; LR CNO, n=4) (Supporting Table 3.2). Rats spent less time exhibiting "stereotyped behaviors" (~5-15% of time; e.g. head movements, circling) (e.g. active port orientation/ approach, immobility) compared to "non-stereotyped behaviors" (e.g. active port orientation/ approach, immobility) (Effect of Category, $F_{1,22}$=9.33, p<0.01) (Fig 3.6). HRs and LRs did not differ on this measure (Effect of Phenotype, $F_{1,22}$=0.22, p=0.65) and treatment did not affect behavior (Effect of Treatment, $F_{1,22}$=0.29, p=0.59) (Supporting Fig S3.7). Thus, in parallel to what was observed in Chapter 3, it appears some animals exhibited stereotypy, which may have interfered with cocaine-induced reinstatement measures. However, the small sample size and lack of significant treatment and phenotype effects warrants further investigation in this regard.

Rate of extinction and reinstatement of drug-seeking behavior

There was not a significant relationship present between rate of extinction (extinction 1) and cue-induced drug-seeking behavior (HR VEH, r^2=0.29, p=0.17; HR CNO, r^2=0.03, p=0.59; LR VEH, r^2<0.001, p=0.99; LR CNO, r^2=0.13, p=0.26), or extinction rate (extinction 2) and cocaine-primed drug-seeking behavior (HR VEH, r^2=0.20, p=0.26; HR CNO, r^2=0.003, p=0.86; LR VEH, r^2=0.06, p=0.43; LR CNO, r^2=0.15, p=0.22). Thus, the variation in drug-seeking behavior during the test for cocaine-primed reinstatement does not appear to be due to differences in extinction rate.

Discussion

In this Appendix, we analyzed data shown in Chapter 3 within the context of the high-responder/ low-responder model. In this model, HRs acquire drug self-administration at a faster rate than LRs (Piazza et al. 1989, Piazza et al. 1990, Piazza et al. 1991, Piazza et al. 1998, Piazza et al. 2000, Klebaur et al. 2001, Mantsch et al. 2001, Ferris et al. 2013), a finding that we replicated here. Although we controlled for the number of infusions received during the acquisition phase, HRs had lower interinfusion intervals and shorter session lengths relative to LRs. HRs also showed greater drug-seeking behavior during the test for cue-induced reinstatement. Although prior studies have reported that HRs and LRs do not differ in relapse propensity (Deroche-Gamonet et al. 2004), methodological differences likely contribute to these seemingly discrepant findings. Perhaps most relevant, in the current study, rats underwent short access cocaine self-administration training that lasted 15 days; whereas the work done by Deroche-Gamonet et al. used a prolonged self-administration schedule that lasted 3-months. We know from studies with the ST/GT animal model, that differences in the timing and duration of the drug self-administration paradigm can be critical to capturing individual differences (or not)

(Saunders and Robinson 2010, Saunders et al. 2013, Kawa et al. 2016). Thus, similar to STs and GTs, HRs and LRs only appear to differ in cue-induced drug-seeking behavior after limited cocaine experience. This is, to our knowledge, the first time outbred HRs and LRs have showed differences in cue-induced reinstatement of drug-seeking behavior.

Inhibition of the PrL-PVT pathway did not affect drug-seeking behavior during cue-induced or cocaine-primed reinstatement in either HRs or LRs. The neurobiological mechanisms underlying individual variation in addiction-related behaviors between HRs and LRs has remained mostly unexplored. Several studies have found that the two phenotypes differentially engage the hypothalamic-pituitary-adrenal axis (Piazza et al. 1991, Kabbaj et al. 2007) and mesolimbic dopamine system (Rouge-Pont et al. 1993, Chefer et al. 2003, McCutcheon et al. 2009, Ferris et al. 2013), and that these differences contribute to differences in addiction-related behaviors between HRs and LRs. Interestingly, inhibition of the PrL-PVT pathway differentially affects cue-induced drug-seeking behavior in the rats in this study when they are classified as STs and GTs, but not when they are classified as HRs and LRs. This further supports the conclusions from Chapter 3, that the PrL-PVT pathway mediates individual variation in the incentive motivational value of a cocaine-cue, and such individual variation is best captured using the sign-tracker/goal-tracker model.

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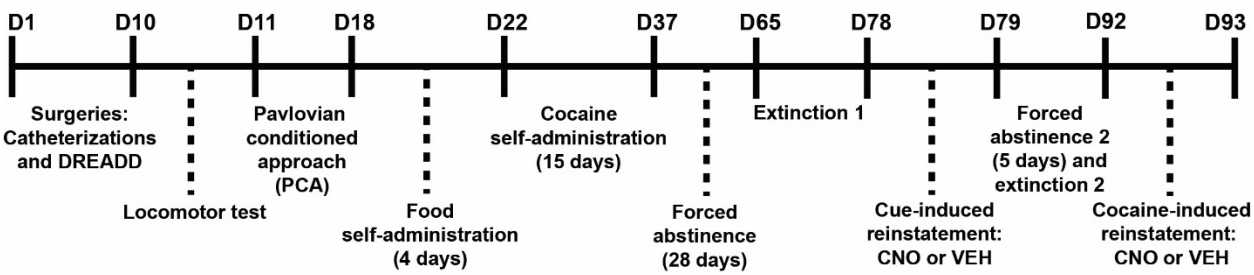
Behavior	Definition
Stereotyped behaviors	
Circling (i.e. “spinning”)	Four consecutive 90° turns occurring within close succession of one another
Head movements	Lateral head movements made when the back paws remain on the flooring and in the same position for a minimum of 2-sec
Non-stereotyped behaviors	
Active port orientation	Oriented toward the active port, but not advancing toward the port
Active port approach	Approached the active port, but made no pokes into the port
Immobility	Time during which all 4 paws remain on the flooring in the same position for a minimum of 2-sec, which no other behaviors co-occurring

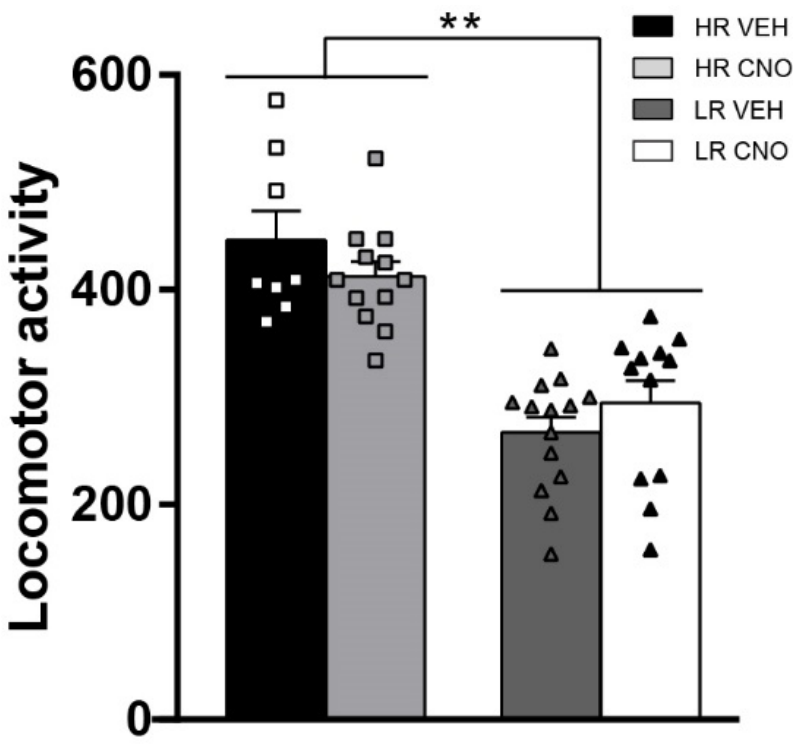
Supporting Table S3.1 Table of behaviors quantified by a blind observer during video analysis of the test for cocaine-primed reinstatement. Behaviors were scored in 30-sec bins every 10-min for the first 60-min of the session. Time spent doing each behavior was recorded per bin.

	Stereotyped behavior			Non-stereotyped behavior		
	Circling	Head movement: no port entry	Head movement: port entry	Immobility	Active port orientation: no movement	Active port approach: no entry
HR VEH (n=5)	2.4% +/- 1.5%	3.3% +/- 3.3%	1.8% +/- 1.3%	4.0% +/- 4.0%	0.0% +/- 0.0%	19.1% +/- 5.1%
HR CNO (n=8)	2.2% +/- 1.1%	0.8% +/- 0.8%	0.6% +/- .06%	3.9% +/- 1.9%	4.1% +/- 2.9%	15.4% +/- 6.0%
LR VEH (n=9)	1.3% +/- 0.7%	4.3% +/- 2.0%	7.5% +/- 6.9%	8.9% +/- 4.6%	0.2% +/- 0.2%	11.9% +/- 3.4%
LR CNO (n=4)	2.0% +/- 2.0%	4.4% +/- 2.5%	3.4% +/- 3.4%	4.2% +/- 3.5%	4.9% +/- 4.9%	11.2% +/- 5.0%

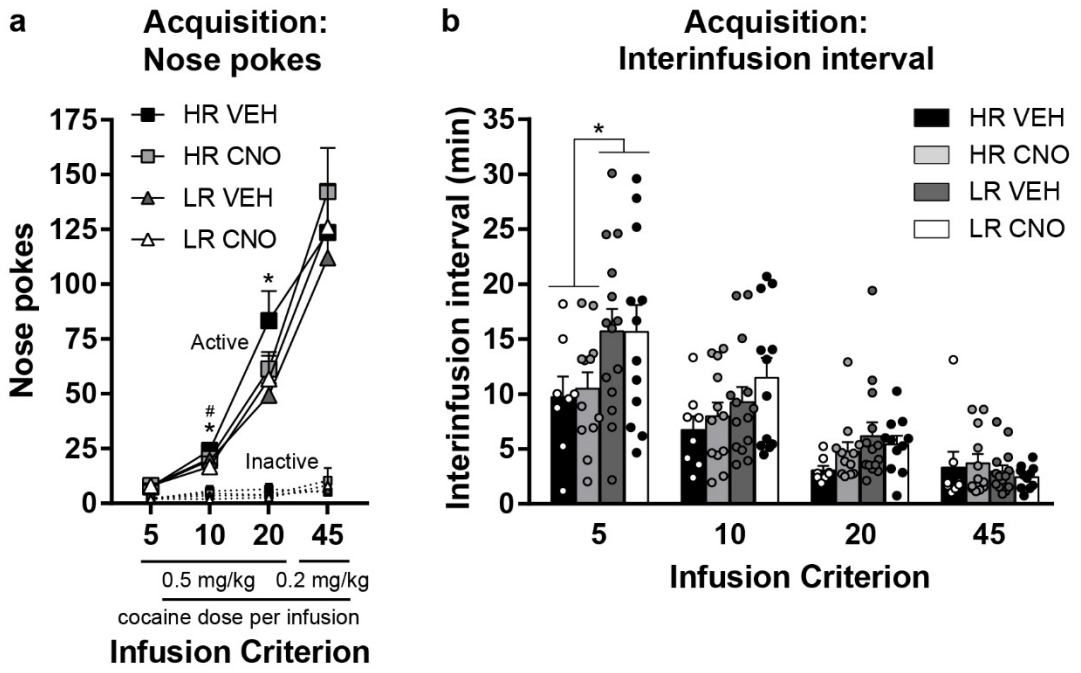
Table S3.2 Percent of time exhibiting stereotyped and non-stereotyped behaviors during cocaine-primed reinstatement. Data are represented as mean percent time +/- SEM. Rats exhibited minimal stereotyped behavior during cocaine-primed reinstatement.



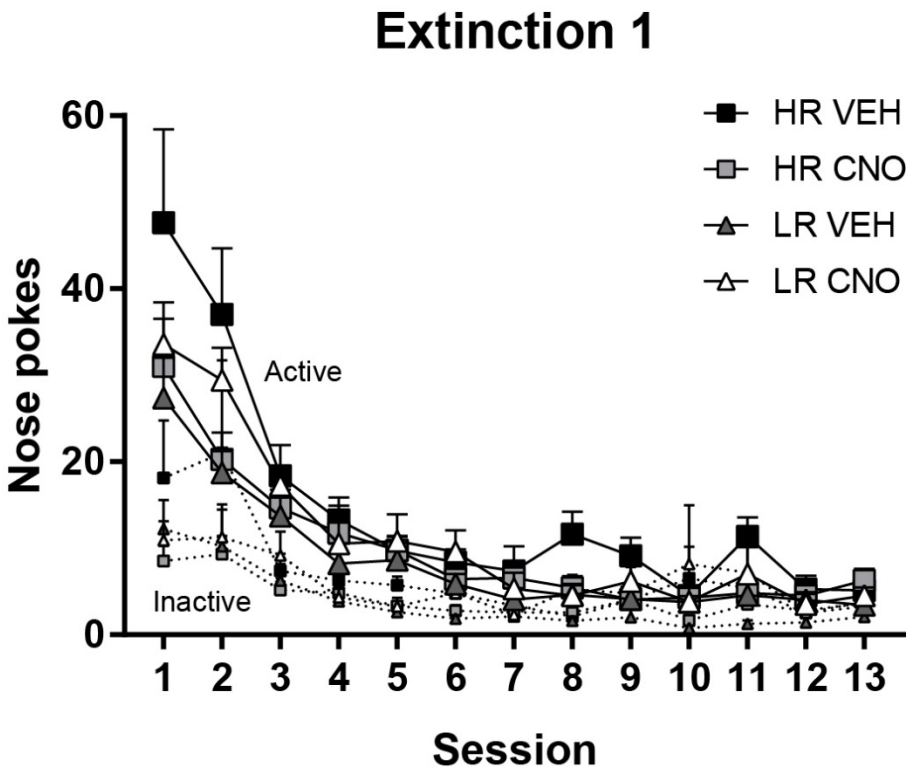
Supporting Fig. S3.1 Experimental timeline. Rats underwent surgeries for indwelling jugular catheter implantation and viral infusions into the PrL, aPVT and pPVT for G_i DREADD expression in the PrL-PVT pathway. Rats were then characterized based on their locomotor response to a novel environment and PCA training. Food self-administration (4 days) and cocaine self-administration (15 days) followed, with a subsequent 28-day forced abstinence period. Daily extinction sessions occurred for 13 consecutive days prior to the cue-induced reinstatement test. Rats were given an injection (i.p.) of either vehicle (VEH) or 5 mg/kg clozapine-N-oxide (CNO) to activate the G_i DREADD prior to the reinstatement test. Following cue-induced reinstatement, rats underwent 5 days of forced abstinence then daily extinction training for 8 consecutive days before cocaine-induced reinstatement. Prior to the reinstatement test, rats were given the same treatment (vehicle or CNO) as that prior to cue-induced reinstatement, as well as a 15 mg/kg injection of cocaine immediately before being placed into the testing chamber.



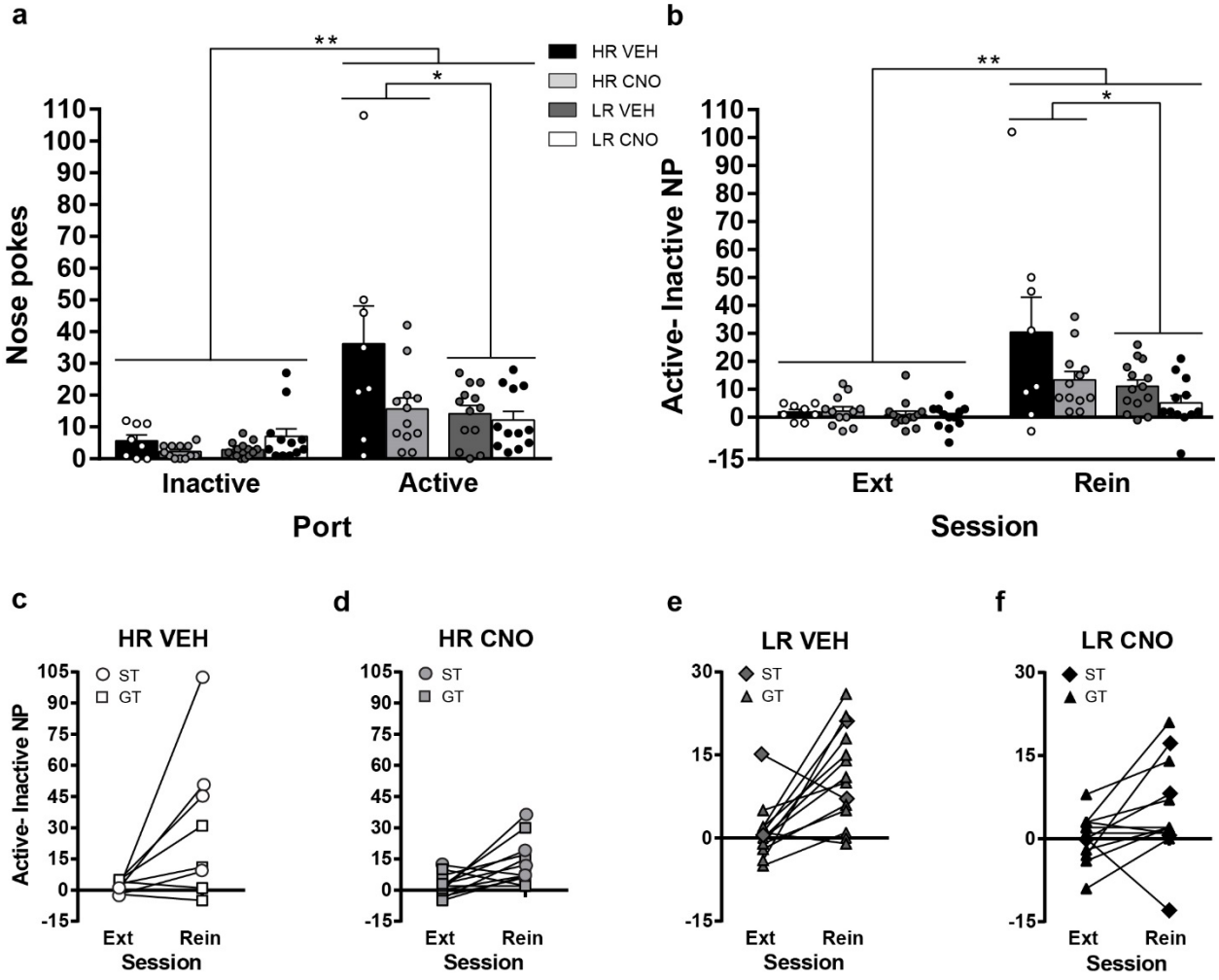
Supporting Fig. S3.2 Locomotor response in a novel environment. Mean + SEM for cumulative locomotor activity in a novel environment. HRs show greater locomotor activity compared to LRs (Effect of Phenotype, $p<0.0001$). Rats are separated into their treatment groups for the test sessions, but did not receive treatment prior to the locomotor test. (HR VEH, n=8; HR CNO, n=12; LR VEH, n=14; LR CNO, n=12) **$p<0.01$



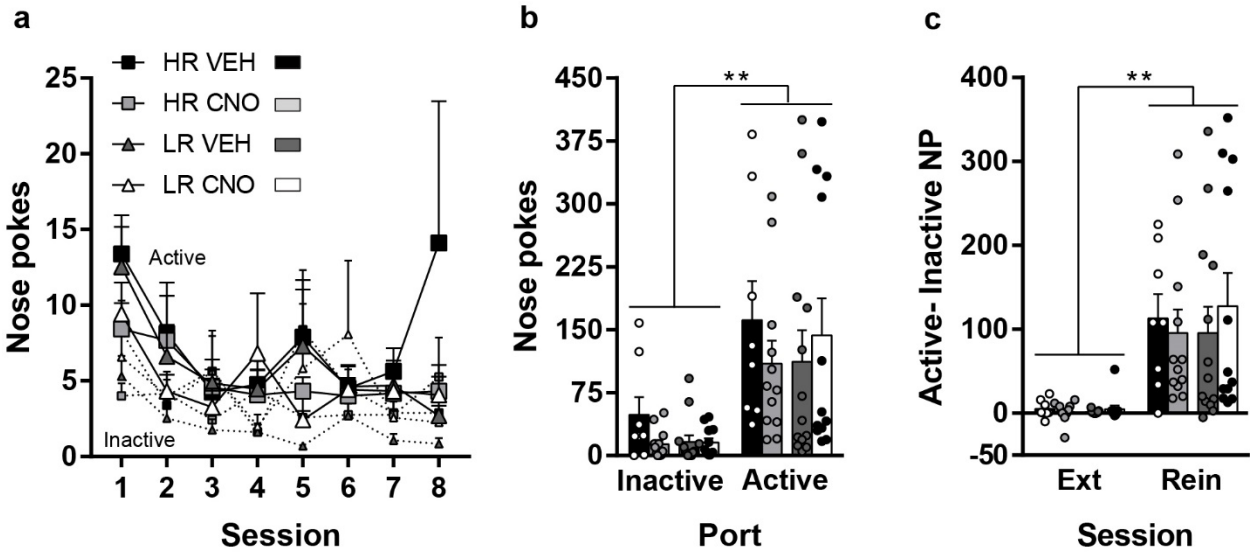
Supporting Fig. S3.3 Acquisition of cocaine self-administration. **a)** Mean + SEM for nose pokes made into the inactive and active port across infusion criterion (IC) for HRs and LRs. All rats made more pokes into the active port ($p<0.0001$) across IC ($p<0.0001$). HRs showed more drug-seeking behavior during IC10 ($p=0.01$) and IC20 than LRs ($p=0.02$). Rats in the vehicle-treated group also showed more drug-seeking behavior during IC10 ($p=0.04$) compared to rats treated with CNO. **b)** Mean + SEM for interinfusion interval at each IC. All rats decreased time between infusions as training progressed ($p<0.0001$) and HRs showed lower interinfusion intervals compared to LRs at IC5 ($p=0.01$). (HR VEH, n=8; HR CNO, n=12; LR VEH, n= 14, LR CNO, n= 12). *HRs different from LRs, $p<0.05$; $^{\#}$Rats in the vehicle group different from rats in the CNO group, $p<0.05$



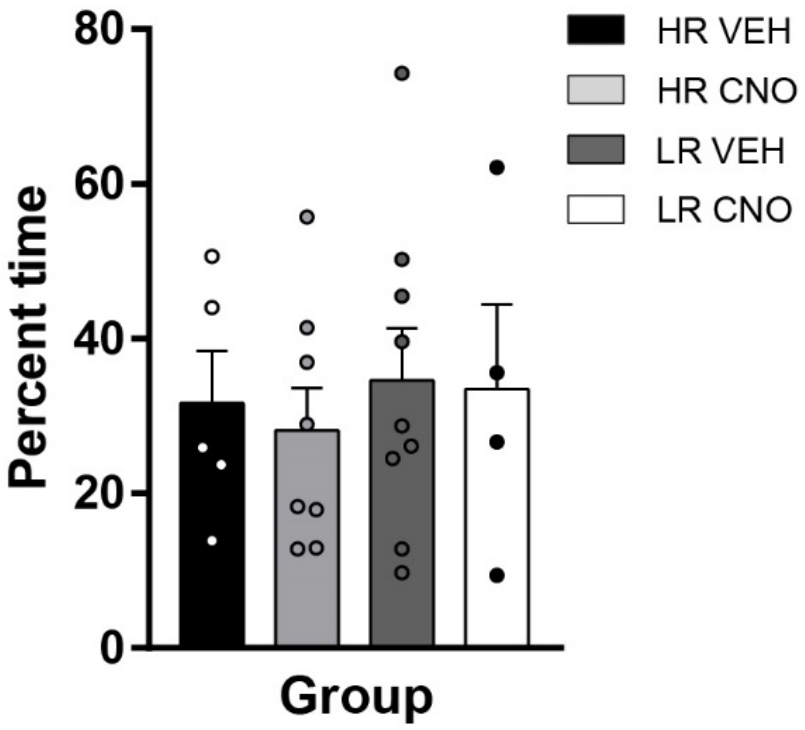
Supporting Fig. S3.4 Extinction training. Mean + SEM for nose pokes made into the inactive and active port across 13 sessions of extinction training. All rats decreased drug-seeking behavior as training progressed ($p<0.0001$). Rats in the HR vehicle group (n=8) showed greater drug-seeking behavior throughout training compared to rats in the LR vehicle group (n=14, $p=0.001$), and HR CNO group (n=12, $p=0.01$). LR rats in the CNO group (n=12) also showed greater drug-seeking behavior compared to rats in the LR vehicle group ($p=0.04$). (HR VEH, n=8; HR CNO, n=12; LR VEH, n= 14, LR CNO, n= 12)



Supporting Fig. S3.5 Effects of chemogenetic inhibition of the PrL-PVT pathway on cue-induced reinstatement of drug-seeking behavior. **a)** Mean + SEM for nose pokes made into the inactive and active port during the test session. There was a significant Effect of Port ($p<0.0001$), Phenotype ($p=0.03$) and Treatment ($p<0.05$). HRs made more pokes into the active port compared to LRs ($p<0.05$). There was a trend toward significance for PrL-PVT inhibition in HRs resulting in less drug-seeking behavior compared to the HR vehicle group ($p<0.07$). **b)** Mean + SEM for active-inactive nose pokes (NP) made during the last extinction training session (Ext) and reinstatement test session (Rein). All rats showed more drug-seeking behavior during the reinstatement session compared to the extinction session (Effect of Session, $p<0.0001$). There was an Effect of Phenotype ($p=0.01$) and Treatment ($p=0.03$). HRs showed greater drug-seeking behavior during the test for reinstatement compared to LRs ($p=0.03$). Individual data points for active-inactive nose pokes (NP) made during the last extinction training session (Ext) and the reinstatement test (Rein) for rats in the **c)** HR VEH (n=8), **d)** HR CNO (n=12), **e)** LR VEH (n=14) and **f**) LR CNO (n=12) group. The symbol of the individual data point indicates if the rat was classified as a sign-tracker (ST) or goal-tracker (GT) based on their behavior during Pavlovian conditioned approach training. *$p<0.05$



Supporting Fig. S3.6. Extinction 2 training and cocaine-primed reinstatement of drug-seeking behavior. **a)** Mean + SEM for nose pokes made into the inactive and active port across 8 sessions of extinction training in HRs and LRs. All rats decreased drug-seeking behavior across training sessions ($p<0.0001$), and behavior did not differ between phenotype (HR or LR) or treatment (VEH or CNO). **B)** Mean + SEM for pokes made into the inactive and active port during a test for cocaine-primed reinstatement. All rats made more pokes into the active port compared to the inactive port (Effect of Port, $p<0.001$). Inhibition of the PrL-PVT pathway did not affect drug-seeking behavior in either phenotype. **C)** Mean + SEM for active-inactive nose pokes (NP) made during the last extinction training session (Ext) and the reinstatement test (Rein). All rats showed more drug-seeking behavior during the reinstatement test compared to the last extinction session (Effect of Session, $p<0.001$). (HR VEH, n=8; HR CNO, n=12; LR VEH, n=13; LR CNO, n=12) **$p<0.01$



Supporting Fig. S3.7 Cocaine-induced stereotyped behavior during cocaine-primed reinstatement. Mean + SEM for percent time exhibiting stereotyped behavior during the first hour of the test for cocaine-primed reinstatement. Behavior did not differ based on phenotype ($p=0.59$) or treatment ($p=0.76$). (HR VEH, n=5; HR CNO, n=8; LR VEH, n=9; LR CNO, n=4)

Chapter 4

General Discussion

The objective of this dissertation was to assess the role of the paraventricular nucleus of the thalamus (PVT) and the cortical projections of the prelimbic cortex (PrL) to the PVT on individual variation in cocaine-seeking behavior. We hypothesized that: 1) The PVT acts as a "brake" to suppress the expression of the incentive motivational value of a cocaine-cue during a test for cue-induced reinstatement; and 2) The PrL-PVT pathway contributes to this function by exerting "top-down" inhibitory control over the PVT and thereby acts to attenuate drug-seeking behavior. Local pharmacology and chemogenentic tools that provided neuroanatomical specificity were used to address these hypotheses. Our results support our first hypothesis, that the PVT acts to inhibit the expression of the incentive motivational properties of the cocaine-cue during cue-induced reinstatement. However, in contrast to our second hypothesis, the PrL-PVT pathway appears to enhance drug-seeking behavior during cue-induced reinstatement and does so selectively in rats that attribute an incentive motivational value to a cocaine-cue (i.e. STs). A detailed discussion of the results for each experiment are addressed in Chapters 2 and 3. The role of the PVT and associated circuitry in cue-reward learning, within the context of addiction-related behaviors, will be further discussed in this Chapter.

The role of the PVT in mediating individual variation in cue-induced reinstatement

Kelley and colleagues (2005) incorporated the PVT as an integral nucleus involved in the hypothalamic-thalamic-striatal axis of the motive circuit over a decade ago (Kelley et al. 2005). The role of the PVT in motivated behaviors has since gained increasing attention. The PVT has been shown to mediate a diverse range of behaviors, including stress and anxiety, fear learning, addiction-related behaviors and cue-reward learning (for review see Hsu et al. 2014, Kirouac 2015, Millan et al. 2017). Work from our lab has identified a role for the PVT in mediating individual differences in incentive salience attribution (Haight and Flagel 2014). Neuronal activation in the PVT increases in STs relative to GTs and an unpaired control group after presentation of a food- (Flagel et al. 2011a) or drug-paired cue (Yager et al. 2015). However, in GTs, there is correlated cue-induced neuronal activity between the PVT and cortical regions; whereas in STs correlated activity is apparent between the PVT and subcortical regions (Flagel et al. 2011a, Haight and Flagel 2014). Thus, it was proposed that the PVT acts as a central node mediating individual variation in incentive salience attribution (Haight and Flagel 2014). This hypothesis was first tested when the effects of excitotoxic lesions to the PVT were assessed for the acquisition and expression of sign- and goal-tracking behavior. Lesions to the PVT prior to the start of Pavlovian conditioned approach (PCA) training increased sign-tracking behavior in GTs and amplified sign-tracking behavior in STs (Haight et al. 2015). When PVT lesions were made after acquisition, sign-tracking behavior in GTs increased, while behavior in STs remained unaffected, likely due to a ceiling effect (Haight et al. 2015). These data suggested that the PVT is acting as a “brake” on the acquisition and expression of incentive salience attribution to a food-cue. Once this brake was released, the incentive motivational value of the food-cue was acquired and/or expressed. Thus, it appears the PVT is acting to attenuate the expression of

incentive salience attribution, and, given the differences in cue-induced "functional activity", it presumably does so via different neural circuit mechanisms in STs and GTs.

The purpose of Chapter 1 was to establish a role of the PVT in mediating individual variation in cue-induced drug-seeking behavior. The PVT is activated in response to drugs of abuse (Deutch et al. 1995, Deutch et al. 1998, Stephenson et al. 1999), and work had shown that inactivation of the posterior PVT (pPVT) attenuates context-induced (Hamlin et al. 2009), cocaine-primed (James et al. 2010), and cue-induced reinstatement of drug-seeking behavior (Matzeu et al. 2015). Work presented in this dissertation (Chapter 2) supports a role of the PVT in mediating individual variation in cue-induced reinstatement of drug-seeking behavior, as transient inactivation of the PVT selectively enhanced cue-induced drug-seeking behavior in GTs (Chapter 2). Thus, the PVT acts to inhibit the incentive motivational value of the cocaine-cue, as releasing this inhibition results in an increase in drug-seeking behavior selectively in GTs, presumably due to the incentive motivational value of the cocaine-cue now being expressed. Thus, the role of the PVT in mediating individual variation in the incentive motivational value of a reward cue is conserved between Pavlovian learning with a natural reward, and instrumental learning with a drug reward, using this ST/GT model.

While the results from Chapter 2 align with our hypothesis, they are not necessarily congruent with those reported in the literature. Prior work has shown that inhibition of the PVT results in a decrease in context-induced (Hamlin et al. 2009), cocaine-primed (James et al. 2010) and cue-induced (Matzeu et al. 2015) drug-seeking behavior. However, these studies only inhibited the pPVT, leaving the anterior PVT (aPVT) intact. The work presented in this dissertation assessed the effects of inactivating the entire rostral-caudal extent of the PVT on cue-induced drug-seeking behavior. While the anterior and posterior regions of the PVT share

considerable overlap in afferent and efferent projections, several differences exist that likely contribute to the discrepancies between our findings and those of others. Most notably, the aPVT projects more densely to the shell subregion of the nucleus accumbens (NAc) compared to core subregion, while the pPVT projects to a similar extent to both NAc subregions (Dong et al. 2017). Recent work by Do-Monte and colleagues (2017) showed a functional dichotomy between the anterior and pPVT during an instrumental test where cue presentation occurred and the sucrose reward was unexpectedly withheld (Do-Monte et al. 2017). Results showed that inhibition of the aPVT, but not the pPVT, enhanced sucrose-seeking behavior during reward omission, and optogenetic activation of the aPVT attenuated reward-seeking behavior under these conditions (Do-Monte et al. 2017). It may be the case, therefore, that the results presented in Chapter 2 of this dissertation are primarily being driven by the aPVT. That is, the aPVT may act to inhibit the expression of the incentive motivational value of the cocaine-cue present in GTs. However, to address this hypothesis, the effects of transient inactivation of the anterior versus posterior regions of the PVT on individual variation in cue-induced reinstatement would have to be assessed.

Do-Monte and colleagues also reported that optogenetic inhibition of the aPVT projections to the NAc shell during reward omission increased sucrose-seeking behavior (Do-Monte et al. 2017). Conversely, inhibition of aPVT projections to the central nucleus of the amygdala (CeA) decreased sucrose-seeking behavior (Do-Monte et al. 2017). Interestingly, these manipulations did not affect behavior when the reward was delivered following cue-presentation as expected (Do-Monte et al. 2017). These data suggest that it is specifically the projections from the aPVT to the NAc shell that act to inhibit continued sucrose-seeking behavior when the action previously resulting in reward delivery no longer does. Inhibition of the aPVT-NAc shell circuit,

therefore, results in an overall disinhibition and associated increase in sucrose-seeking behavior. It is possible, therefore, that the increase in drug-seeking behavior selectively in GTs found in Chapter 2 is a result of disinhibition of aPVT projections to the NAc shell as a function of global PVT inactivation. Work to date, however, has shown that GTs do not engage the neurons projecting from the PVT to NAc in response to a food cue , and this is true of both anterior and posterior subregions of the PVT (Haight et al. 2017). Yet, it is possible that different patterns of neuronal activation would emerge in response to a cocaine-cue following cocaine self-administration and abstinence, and this remains to be addressed in STs and GTs. To determine whether STs and GTs engage different cell populations within the PVT, one could use TRAP (Targeted Recombination in Active Populations) technology following exposure to the cocaine-associated cue that elicits reinstatement of drug-seeking behavior. In this approach, transgenic mice express CreER in activated cell populations allowing for the identification of neuronal ensembles involved in specific behaviors (for review see DeNardo et al. 2017). While this technology is not currently available in rats lines, mice have been shown to exhibit sign- and goal-tracking behavior (Campus et al. 2016), and could potentially be used to exploit the TRAP technology.

PVT circuity mediating motivated behavior

As discussed in Chapter 1, GTs engage “top-down” cortico-thalamic (PVT) circuitry in response to a food-cue, whereas STs engage “bottom-up” hypothalamic-thalamic (PVT)-striatal circuitry (Haight et al. 2017). However, both phenotypes engage cortical projections from the PrL to the PVT in response to a food-cue, and we hypothesize that this pathway acts to inhibit the incentive motivational value of a reward cue. In GTs, this pathway appears to guide

behavior; whereas in STs subcortical “overdrive” may mask these cortical processes, resulting in enhanced incentive salience attribution to reward cues. We believe it is this imbalance between “top-down” and “bottom-up” processing that resulting in the goal-tracking and sign-tracking conditioned responses. The objective of Chapter 3 was to assess the role of the PrL-PVT pathway in individual variation in cue- and cocaine-primed reinstatement. These results from Chapter 3 oppose our hypothesis that the PrL-PVT pathway acts to inhibit the incentive motivational value of the cocaine-cue, and this discrepancy is discussed in the following sections in the context of PVT-associated circuitry.

Cortical projections to the PVT

The prefrontal cortex (PFC) is a region well known for mediating motivated behaviors and the PFC neurocircuitry underlying these behaviors has been thoroughly explored (Matsumoto et al. 2003, Kouneiher et al. 2009, Warden et al. 2012, Moorman et al. 2015). Recently, projections from the PFC to the PVT have been associated with mediating appetitive reward-seeking behavior during Pavlovian learning in mice (Otis et al. 2017). In this study, mice learned to discriminate between a conditioned stimulus that predicted subsequent sucrose-reward delivery (CS+) and a conditioned stimulus that did not predict reward delivery (CS-). After learning the association between the reward and CSs, mice were head-fixed and in vivo two-photon calcium imaging was used to assess neuronal activity in PFC projections to the PVT in response to the CS+ and CS-. As the mice were head-fixed, conditioned reward-seeking was measured as anticipatory licks in response to the CS+. The pathway did not respond to presentation of the CS-, but showed an increase in neuronal inhibition in response to the CS+ (Otis et al. 2017). Furthermore, optogenetic inhibition of this pathway increased conditioned

reward-seeking behavior in response to the CS+, while activation had the opposite effects (Otis et al. 2017). These data suggest that the PFC-PVT pathway acts to inhibit conditioned reward-seeking to a natural reward, as inhibition of this pathway results in an overall disinhibition of the pathway and an increase in reward-seeking behavior. While this study did not specifically target the PrL, the dorsomedial cells in layer VI of the cortex were targeted, which is the location of the PrL neurons projecting to the PVT (Li and Kirouac 2012). Work from our lab expands upon this notion that cortical projections to the PVT mediates appetitive Pavlovian behavior. Selective chemogenetic inhibition of this pathway increases sign-tracking behavior in GTs, but does not affect behavior in STs (Campus et al. 2018). Conversely, activation of this pathway increases goal-tracking behavior in STs, without affecting behavior in STs (Campus et al. 2018). Together, these data suggest that cortical projections to the PVT mediate Pavlovian cue-reward learning, and specifically, individual variation in the motivational value of the reward cue.

Based on these findings, and those presented in Chapter 2 of this dissertation (Kuhn et al. 2018), we hypothesized that the PrL-PVT pathway would have the same function in the context of a cocaine-cue mediating individual variation in cue-induced reinstatement. That is, this pathway would act to attenuate the incentive value of the cocaine-cue in GTs, and inhibiting this pathway would result in an overall disinhibition and an increase in drug-seeking behavior selectively in GT (as did inactivation of the PVT; Kuhn et al. 2018). Our results, however, were in direct opposition to our hypothesis; as cue-induced drug-seeking behavior in GTs was unaffected, while drug-seeking in STs was attenuated. This discrepancy between our hypothesis and the results discussed in Chapter 3 may be due to the PrL-PVT undergoing cocaine-induced neuroplastic changes.

Neuroplasticity within the motive circuitry, including cortico-thalamic-striatal circuits, has been explored following drug experience and varying periods of abstinence (for review see Nestler 2001, Robinson and Kolb 2004, Kalivas et al. 2008). Changes in dendritic spine density in the NAc shell have be shown following a single injection of amphetamine and a brief (2-3 day) abstinence period (Kolb et al. 2003). However, these changes are minimal compared to the effects of repeated injections of amphetamine (9 days) prior to a three-week abstinence period (Kolb et al. 2003), suggesting that continuous experience with a drug and a longer period of abstinence further enhances neuroplastic changes. In support, after several weeks of repeated injections of amphetamine (Robinson et al. 1997) or cocaine (Robinson et al. 1999), and a three week abstinence period, drug-induced alterations in dendritic morphology (spine density, dendritic length, etc.) are evident on neurons within the NAc and the PFC. Other neuromolecular changes within the mesocorticolimbic dopamine system have been reported over the course of 30-90 days of forced abstinence following relatively limited (10 days) cocaine self-administration experience (Lu et al. 2003). These include drug-induced increases in BDNF (brain-derived neurotrophic factors) levels, a growth factor that has been specifically associated with neuroplasticity (Grimm et al. 2003). PFC projection neurons to the NAc have also been shown to change during forced abstinence, such as a decrease in the coupling of PFC dopamine 2 receptors and inhibitory G-protein-coupled-receptors (Bowers et al. 2004). This molecular change, among others, are suspected to contribute to changes in NAc glutamatergic signaling associated with reinstatement of drug-seeking behavior following abstinence (for review see Kalivas et al. 2005, Bobadilla et al. 2017). There is also recent evidence supporting drug-induced changes in the PrL-PVT circuitry that may be associated with the reinstatement of drug-seeking behavior (Fig. 4.1). Neuronal activation (i.e. c-fos expression) in layer VI of the PrL (location of

PrL neurons projecting to the PVT) and in the pPVT is elevated 2-hr after the final session of a 2-week cocaine self-administration paradigm, suggesting this pathway is engaged following brief cocaine experience (Giannotti et al. 2018). In the same report, it was shown that inhibition of the PrL-PVT pathway after the conclusion of cocaine self-administration attenuated cue-induced drug-seeking behavior, suggesting that the immediate changes in this pathway following cocaine self-administration training contribute to the reinstatement of drug-seeking behavior (Giannotti et al. 2018). The results presented in Chapter 3 of this Dissertation are seemingly congruent with these findings, as we demonstrate a decrease in cue-induced drug-seeking behavior following inhibition of the PrL-PVT pathway. Importantly, however, our reported effects were specific to STs and occurred following one month of forced abstinence and extinction training procedures. Thus, drug-induced changes in the PrL-PVT circuit appear to occur early after cocaine experience and persist over prolonged periods of abstinence. The mechanisms underlying these neuroplastic alterations presumably differ depending on the time point of evaluation and phenotype under study; but, together, these results suggest that cocaine acts directly on the PrL-PVT circuit, which then plays a role in relapse propensity.

Nucleus accumbens

The mechanisms by which the PrL-PVT projection attenuates cue-induced drug-seeking behavior remains to be explored (Fig 4.1). The PVT sends dense glutamatergic projects to the nucleus accumbens (NAc) (Christie et al. 1987, Li and Kirouac 2008, Vertes et al. 2008), with greater innervation of the shell subregion compared to the core subregion (Dong et al. 2017). The NAc has been shown to be critical in mediating addiction-related behaviors (for review see Hikida et al. 2016, Scofield et al. 2016, Cooper et al. 2017). Excitation of the PVT along the

rostral-caudal axis increases dopamine metabolites within the NAc (Jones et al. 1989), and the PVT can elicit dopamine release within the NAc independent of the VTA (Parsons et al. 2007, Perez et al. 2018). Projections from the PVT to the NAc shell also terminate in close proximity to dopamine neurons (Pinto et al. 2003, Parsons et al. 2007). It is believed that PVT projections stimulate dopamine release within the NAc by acting on ionotropic glutamate receptors on the dopamine fibers from the ventral tegmental area resulting in dopamine release (Parsons et al. 2007). Given the influence the PVT can have on dopamine release within the NAc, it is not surprising that the PVT-NAc pathway has been implicated in mediating several forms of addiction-related behaviors. Recent work has shown that disrupting neuronal transmission between the PVT and NAc shell decreases cocaine self-administration (Neumann et al. 2016). The PVT-NAc pathway also undergoes neuronal plasticity during forced abstinence following cocaine self-administration (Joffe et al. 2016, Neumann et al. 2016). Within the first few days of forced abstinence, AMPA and NMDA receptor levels become enhanced and it is hypothesized this leads to an increase in GluN2B receptors in the NAc core and of GluN2C/D receptors in the NAc shell (Joffe and Grueter 2016, Neumann et al. 2016). Excitatory transmission between the PVT and NAc shell medium spiny neurons are also potentiated following morphine exposure, and this pathway mediates opioid withdrawal symptoms, as inhibition attenuates aversive withdrawal symptoms (Zhu et al. 2016). These data are especially interesting, as it appears the PVT-NAc pathway mediates both appetitive and aversive motivational states, suggesting different cell populations within the PVT-NAc pathway may be involved depending on the affective state. Lastly, cells projecting from the entire rostral-caudal axis of the PVT to the NAc shell subregion are engaged during context-induced reinstatement (Hamlin et al. 2009). Thus, the

projection from the PVT to the NAc appears to be involved in multiple steps of addiction, from drug-taking behavior, to withdrawal and drug-seeking behavior (Fig 4.1).

It is possible that inhibition of the PrL-PVT pathway affects downstream communication between the PVT and NAc. In fact, recent work from our lab shows that chemogenetically manipulating the PrL-PVT pathway affects extracellular dopamine levels within the NAc shell (Campus et al. 2018). While the majority of the work focusing on the contribution of the PVT-NAc pathway on addiction-related behaviors has focused on the shell subregion of the NAc, the entire rostral-caudal axis of the PVT sends projections to the core subregion of the NAc (Dong et al. 2017). The aPVT projects predominantly to the NAc shell, whereas the pPVT sends comparable projections to both subregions of the NAc. This is important to consider, as most work to-date has focused on the role of the pPVT in context-induced (Hamlin et al. 2009), cue-induced (Matzeu et al. 2015, Matzeu et al. 2016) and cocaine-primed (James et al. 2010) reinstatement of drug-seeking behavior. Additionally, projections from the pPVT to the NAc "shore" (area between the shore and core) are also engaged in STs in response to a reward cue, suggesting that this pathway does in fact mediate incentive salience attribution (Haight et al. 2017). As previously discussed, sign-tracking behavior is dependent upon dopamine signaling within the NAc core while goal-tracking behavior is dopamine-independent (Flagel et al. 2011b, Saunders and Robinson 2012). Moreover, blocking dopamine transmission within the NAc core attenuates cue-induced drug-seeking behavior in STs, suggesting dopamine signaling within this region contributes to the incentive value of the cocaine-cue (Saunders et al. 2013). Thus, the results described within this dissertation may very well have been the result of affecting activity (e.g. dopamine transmission) within the NAc as a function of changes in PVT activity. Chemogenetic or optogenetic tools would be ideal to assess the functional role of the PVT-NAc

pathway in reinstatement behavior. Additionally, microdialysis can be used to determine if PrL-PVT inhibition affects extrasynaptic dopamine levels within the NAc core.

PVT projections to the PrL

While the PVT receives projections from the PrL, it also sends reciprocal projections back to the PrL, although this projection is not as dense as that to the NAc (Li and Kirouac 2008, Dong et al. 2017). Work has shown that several PVT cells that project to the NAc bifurcate and also project to the PrL (Bubser et al. 1998, Otake et al. 1998). It is possible that inhibition of the PrL-PVT pathway can then result is reciprocal inhibition of cells within the PrL. While the role of the PrL in drug-seeking behavior has been debated (for review see Moorman et al. 2015), inactivation of the PrL prior to cue-induced reinstatement decreases drug-seeking behavior (Stefanik et al. 2016, Giannotti et al. 2018). While the functional role of the PrL in sign- and goal-tracking has yet to be assessed, PFC neurochemistry differs between STs and GTs in response to a Pavlovian cocaine-cue, such that extrasynaptic dopamine levels increase, but only in STs (Pitchers et al. 2017b). Indeed, in STs, cocaine cue-induced dopamine levels in the PFC are positively correlated with approach to the cocaine-cue. While cocaine-cue-induced dopamine levels were not affected in GTs (Pitchers et al. 2017b), acetylcholine levels were increased in response to the cocaine-cue in GTs, but there was no correlation with behavior (Pitchers et al. 2017b). These data suggest that cortical dopamine levels contribute to incentive salience attribution, and that neurotransmitters within the PFC differentially mediate cue-reward learning between STs and GTs. It is possible that PrL-PVT inhibition, in addition to affecting transmission within the NAc, may also inhibit activity (e.g. dopamine levels) within the PrL via

reciprocal connections, resulting in a decrease in cue-induced drug-seeking behavior selectively in STs (Fig 4.1).

Lateral hypothalamus

Orexinergic fibers originating from the lateral hypothalamus (LH) send projections to the PVT (Peyron et al. 1998, Parsons et al. 2006), and, as discussed in Chapter 1 of this dissertation, the LH is considered a key component of the motive circuitry (Kelley et al. 2005). Sign-trackers show enhanced cue-induced neuronal activation in LH projections to the PVT, suggesting a role of this pathway in incentive salience attribution (Haight et al. 2017). Work from our lab has shown that antagonizing either orexin-A or orexin-B receptors selectively in the PVT decreases sign-tracking behavior, presumably by attenuating the incentive motivational value of the reward cue (Haight 2016, Campus et al. 2017). Ongoing work in our lab is assessing the density of orexin-A and orexin-B receptors in the PVT to better elucidate the role of orexin in the PVT in mediating sign- and goal-tracking behavior. Orexinergic signaling has also been associated with addiction-related behaviors, including drug-seeking behavior (for review see Mahler et al. 2012, Mahler et al. 2014, James et al. 2017). Antagonizing orexin-A receptors within the pPVT attenuates cue-induced drug-seeking behavior, while local administration of orexin enhances drug-seeking behavior (Matzeu et al. 2016). However, orexin-induced enhancement of cue-induced drug-seeking behavior is abolished in the presence of dynorphin, suggesting a role of the kappa-opioid system in mediating orexinergic transmission within the PVT (Matzeu et al. 2018). While it is unknown if the cortical cells projecting from the PrL to the PVT synapse onto cells containing orexin receptors, this is one potential mechanism by which the PrL-PVT may exert its "inhibitory" top-down control. That is, the PrL-PVT circuit may act to attenuate orexinergic

signaling within the PVT, resulting in a decrease in the incentive motivational value of the cocaine-cue in STs and thus an attenuation of cue-induced drug-seeking behavior (Fig 4.1). To better asses this, in vivo electrophysiology may be used to determine if PrL-PVT inhibition affects the electrochemical properties of neurons originating in the LH. Histological techniques could also be employed to determine if PrL afferents in the PVT synapse onto, or in close proximity, to cells expressing orexin receptors.

Self-administration paradigms and modeling addiction

The work contained within this dissertation allowed rats to self-administer cocaine for approximately 2-weeks during daily 3-hr sessions, where the number of infusions per session were controlled for. Self-administration training sessions that last 1-3 hours are known as short-access (ShA) paradigms (Ahmed et al. 1998, Grimm et al. 2001, Mahler et al. 2014, Smith et al. 2014). However, in contrast to the experiments contained within this dissertation, during ShA training paradigms rats are typically given unlimited access to the drug (i.e. no infusion criterion instituted). While ShA training is commonly used, its relevance for modeling addiction-related behaviors has been challenged with research suggesting that extending the session length to 6-hrs (long-access training, LgA) results in behavior more applicable to the transition to addiction (Ahmed and Koob 1998). Compared to ShA, rats undergoing LgA cocaine self-administration training show an increase in drug-taking behavior (Ahmed and Koob 1998, Mantsch et al. 2004, Mandt et al. 2015), have a higher cocaine break-point (Paterson et al. 2003, Hao et al. 2010), and greater drug-seeking behavior during cocaine-primed reinstatement (Mantsch et al. 2004, Knackstedt et al. 2007). In addition to focusing on the length of the training session, the temporal pattern of drug delivery during training has recently been recognized as an important factor to

consider when modeling the transition to addiction (Zimmer et al. 2012). Work in humans has shown that experienced cocaine users take cocaine intermittently. That is, a large quantity of cocaine is initially consumed during a short time period, followed by a long period with no drug use, before using cocaine again in a similar temporal pattern (Beveridge et al. 2012, Allain et al. 2015). It has been suggested that this intermittent cocaine consumption behavior results in a constant spiking in brain-cocaine concentration levels, which, in turn, elicits addiction-related behavior (Zimmer et al. 2012). Indeed, rats trained to self-administer cocaine using intermittent access (IntA) during daily 6-hr sessions show greater motivation to self-administer cocaine relative to rats in a ShA or LgA group, even though rats in the LgA group administer more cocaine (Zimmer et al. 2012). Interestingly, neither the motivation to self-administer cocaine, nor drug-seeking behavior during a test for cocaine-primed reinstatement, differ between rats trained using IntA during daily 2-hr or 6-hr training sessions (Allain et al. 2018). This suggests that the pharmacokinetics of drug delivery may be more important than the length of the session for modeling the transition to addiction.

Previous work has shown that when a prolonged (36 days) IntA training schedule (sessions lasting 4-hr) is used, STs and GTs do not differ in the motivation to self-administer cocaine (Kawa et al. 2016). At the conclusion of this prolonged intermittent training paradigm, rats underwent a test for cocaine-primed reinstatement, followed by extinction training (5 days) and a test for cue-induced reinstatement. STs and GTs did not differ in drug-seeking behavior during either test of reinstatement following prolonged intermittent access to cocaine (Kawa et al. 2016). However, there was not a period of forced abstinence used in the Kawa et al. study, as has been done in previous studies showing individual variation in relapse propensity between STs and GTs (Saunders and Robinson 2010, Saunders and Robinson 2011, Saunders et al. 2013).

As others have shown that the length of a forced abstinence period is critical factor in determining the degree to which rats will exhibit cue-induced relapse behavior (Grimm et al. 2001, Grimm et al. 2002), it will be important to assess whether individual variation in relapse propensity is evident following the prolonged intermittent access procedure with a subsequent forced abstinence period. In fact, as reported in this dissertation, individual variation in cue-induced reinstatement of drug-seeking behavior was evident following a 28-day forced abstinence period (Chapter 3), but not a 14-day period (Chapter 2).

While the work presented in this dissertation used a ShA schedule and did not employ IntA, the findings are still pertinent for modeling addiction-related behavior. First, all rats acquired cocaine self-administration and increased drug-seeking behavior as infusion criterion increased, demonstrating a continued motivation to take cocaine (Chapter 2 and 3). Second, all rats exhibited cue-induced reinstatement of drug-seeking behavior following a period of forced abstinence (Chapters 2 and 3). Thus, the paradigm we used was effective at both promoting drug-taking behavior and evoking relapse, both of which are characteristic traits of addiction. The discrepancies noted in individual variation in relapse propensity in STs and GTs following ShA versus prolonged IntA to cocaine suggest that the ST/GT model best captures individual variation in the early stages of addiction; that is, after relatively limited drug-taking experience, at which point there are differences in the motivation to work for cocaine (Saunders and Robinson 2011) and in relapse propensity (Saunders and Robinson 2010, Saunders and Robinson 2011, Saunders et al. 2013). Taken together, the behavior of sign-trackers is reflective of one pathway of addiction vulnerability which is apparent in the early stages of drug-taking behavior, yet no less critical than those that emerge following prolonged drug exposure.

Concluding remarks and future directions

The experiments presented within this dissertation sought to elucidate the role of the PVT and associated cortico-thalamic circuitry in mediating individual variation in drug-seeking behavior. We expanded upon previous findings from our lab and established a role for the PVT in mediating individual variation in cue-induced reinstatement of drug-seeking behavior. We also highlighted the need to assess the function of both the anterior and posterior subregions of the PVT in cue-induced drug-seeking behavior, as our results contrast those selectively assessing the role of the pPVT in drug-seeking behavior. Furthermore, our data suggests that the PrL-PVT pathway acts to enhance the incentive motivational value of the cocaine-cue selectively in STs. These results are likely due to downstream effects of PrL-PVT inhibition on dopamine transmission within the NAc. Together, the data presented within this dissertation address the importance and complex nature of the PVT and its associated circuitry in mediating individual variation in cue-induced reinstatement of drug-seeking behavior.

Future studies should further address the role of specific PVT pathways in mediating individual variation in cue-reward learning. Current work in our lab is exploring the role of the LH-PVT pathway as well as the PVT-NAc pathway in mediating sign- and goal-tracking behavior. In doing so, anatomical specificity focused on anterior or posterior subregions of the PVT should be considered. Previous work has shown that the conditioned reinforcing properties of a nicotine (Yager et al. 2015) and opioid-paired cue (Yager et al. 2015) are greater in STs versus GTs. However, individual variation in the conditioned reinforcing properties of these cues (i.e. cue-induced reinstatement) have not been assessed after instrumental drug self-administration, forced abstinence and extinction training. Specifically, assessing the role of the PVT in mediating drug-seeking behavior using different drugs of abuse would greatly enhance

our knowledge of the role of the PVT and its associated circuitry in mediating addiction-like behaviors, specifically within the context of individual variation in reinstatement behavior.

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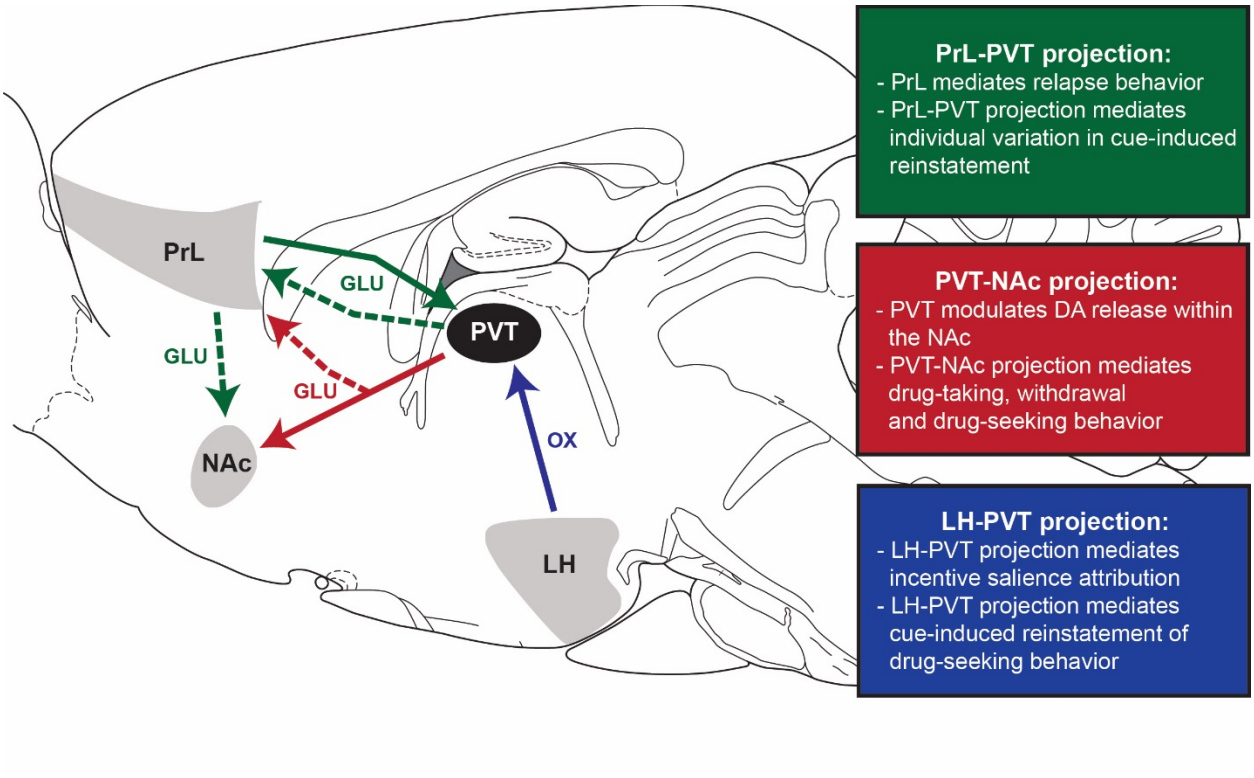

Fig. 4.1 Schematic illustrating paraventricular nucleus of the thalamus (PVT) circuitry mediating addiction-relative behavior. The prelimbic cortex (PrL) sends glutamatergic (Glu) projections to the nucleus accumbens (NAc) as well as the PVT, which sends reciprocal Glu projections back to the PrL. A dense glutamatergic projection also exists between the PVT and the NAc, and this pathway can directly modulate dopamine (DA) release in the NAc. The lateral hypothalamus (LH) sends dense orexinergic (OX) projections to the PVT. As discussed in the main text of this Chapter, these pathways have been shown to contribute to different aspects of addiction-related behaviors, including drug-taking behavior, withdrawal and relapse.