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DEVELOPMENT OF AN AVIAN MODEL FOR IDENTIFYING INDIVIDUAL DIFFERENCES IN DRUG VULNERABILITY

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THESIS

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ABSTRACT OF THESIS

DEVELOPMENT OF AN AVIAN MODEL FOR IDENTIFYING INDIVIDUAL DIFFERENCES IN DRUG VULNERABILITY

The attribution of incentive salience to cues that become associated with drugs of abuse is a critical characteristic of individuals who may be vulnerable to drug addiction. Rodents with the propensity to sign track are thought to be vulnerable to drug abuse. The goal of the current work was to investigate whether sign trackers (STs) would acquire cocaine conditioned place preference (CPP) to a discrete cue using an avian species. In Experiment 1, sign and goal trackers (GTs) were first identified using a one third rank order split. Following identification, cocaine-CPP was conducted with a discrete cue in each end chamber. Contrary to previous research, results showed that GTs showed a CPP to the discrete cue but STs did not. Experiment 2 was conducted to determine whether sign and GTs had been misclassified with the rank order split. Experiment 2 compared the rank order method with a t-test method (absolute criterion). Misclassification of both sign and GTs occurred using the rank order split. The findings indicated that use of a more accurate method to identify sign and GTs may have led to different results for Experiment 1. The t-test method may be useful for models that require identification of STs.

KEYWORDS: Incentive salience, drug abuse, sign and goal tracker (STs, GTs), cues, conditioned place preference (CPP)

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_____June, 16, 2015___ Date

DEVELOPMENT OF AN AVIAN MODEL FOR IDENTIFYING INDIVIDUAL DIFFERENCES IN DRUG VULNERABILITY

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_____April 27, 2015_____

Dedicated to my daughter, Abigail Katherine Osterholm

You have taught me to never give up
"The most certain way to succeed is to try just one more time" *Thomas Edison*

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Cocaine is one of the most frequently abused drugs, with an average annual use of 1.9 million current and past users in the US alone (Taylor, Lewis, & Olive, 2013; NSDUH, 2013). Of current cocaine users, 2.7% began using as early as twelve grade, with the majority of them (1.4 million) meeting the DSM-IV criteria for addiction (Coalition against drug abuse, 2012; NSDUH, 2012). One in every four visits to the emergency room for drug misuse is due to cocaine abuse (Drug abuse warning network, DAWN, 2012). Currently, there are no FDA approved pharmacological treatments for cocaine abuse. Addiction to cocaine has been characterized as a chronic relapsing disease, with 40-60 percent of individuals who seek treatment for drug abuse continuing to relapse. (Milton & Everitt, 2012; National institute of health (NIH), 2012).

One of the biggest problems in treating drug addiction may be relapse. Cues (items, places or people) associated with a drug may trigger relapse (Lee, Milton & Everitt, 2006; Saunders & Robinson, 2013; Meyer, Ma & Robinson, 2012b). In the absence of drugs, physical withdrawal symptoms, established from chronic drug taking may induce drug seeking in the presence of these cues (Solomon & Corbit, 1974; Lee et al., 2006; Saunders &Robinson, 2013). Without the drug, withdrawal is experienced and the ensuing withdrawal escape (behavior to minimize negative physical state of withdrawal) may yield renewed drug taking behavior (Everitt, Belin, Economidou, Pelloux, Dalley & Robbins, 2008; Milton & Everit, 2012; Cardinal, Pennicott, Sugathapala, Robbins & Everitt, 2001; Solomon & Corbit, 1974). In recent studies with human participants it has been demonstrated that cues associated with drugs of reward instigate drug seeking

(Wang, Shi, Chen, Xu, Li, Sun & Lu, 2013; Seo, Lacadie, Tuit, Hong, Constable & Sinha, 2013). Specifically, studies investigating cocaine cues established that subjects experience increased craving in response to cocaine-related cues. (Fox, Tuit & Sinha, 2012). In animal models, it has been reliably demonstrated that cues paired with drugs of reward may initiate renewed drug seeking behavior (Loweth, Tseng & Wol 2013; Pratt & Ford, 2013; Fischer, Houston & Rebec, 2013; Gipson et al., 2013). Collectively, these experiments suggest that cue triggered relapse may be one of the underlying problems with drug abuse.

Cocaine Mechanisms

Cocaine is derived from the leaves of a coca plant and manufactured as either hydrochloride (powder) or hydrochloride with bicarbonate (crack) (Iversen et al., 2009). Cocaine as a powder is generally snorted, while crack is typically smoked or injected with a half-life of 60 min (Iverson et. al., 2009). Absorption of cocaine is rapid, with injection or snorting taking less than three min and smoking as little as 10 sec (Iverson et al., 2009). Cocaine is excreted through urine in 8 to 12 hr from time of administration (Volkow et al., 1993).

The distribution of cocaine is widespread, up regulating dopamine (DA), serotonin (5-HT) and norepinephrine (NE) by way of blocking reuptake in the brain (Iverson, et al, 2009). Cocaine administration results in an increase of DA in the mesocorticolimbic dopamine system, via the blockade of the reuptake DA transporter (DAT) (Xi et al., 2012; Furst, Riba & Al-Khrasani, 2013). After administration of cocaine, there is also increased

glutamate expression in the nucleus accumbens (NAc) and increased dopamine expression in the ventral tegmental area (Xi et al., 2012; Furst et al., 2013).

Cocaine's primary sites of action are the presynaptic monoaminergic terminals of the NAc and the dorsal striatum in the mesocorticolimbic DA system (Jonkman & Kenny 2013; Shalev, Grimm & Shaham, 2002). The NAc is responsible for the rewarding effects of cocaine and after repeated or escalated doses, the dorsal striatum is implicated in habitual behaviors associated with cocaine (Jonkman et. al., 2013). Cocaine in the NAc works as an inhibitor of the NE, DA, 5-HT via the reuptake transporters; NET, DAT, and SERT (Iverson et al., 2009). Blockade of the transporters increase the extracellular concentration of NE, DA and 5-HT, which contributes to the euphoric high of the drug. This is accomplished by reversing the transporter function of NE, DA and 5-HT by flushing out the entire transmitter into the cytosol per VMAT2 (Taylor et al., 2013). Reinforcing effects of cocaine are related to the cocaine-induced release of DA in the mesocorticolimbic dopamine system (Koob & Nester, 1997). While DA has a stronger relationship with the rewarding aspects of cocaine than that of NET, and SERT blockade, all contribute to the rewarding effects of cocaine (Sora et al., 2001).

Cocaine-related Neurotransmitters

Dopamine. Dopamine is a catecholamine of the central nervous system (CNS) (Iverson et al., 2009). There are five known dopamine receptor subtypes in mammals: D_1 through D_5. All of these receptor subtypes fall under two categories, D_1 (comprised of D_1 and D_5) and D_2 (comprised of D_2, D_3 and D_4) (Jonkman et. al., 2013). There are three main fiber systems in the CNS with many more dopamine containing nuclei (Iverson et al.,

2009). The *ultrashort system* projects to the olfactory bulb. The *intermediate-length* system projects to the pituitary, tuberoinfundibular system, and hypothalamus vagues nerve. The *long* system projects to the ventral tegmental, substantia nigra, neostriatum and various other limbic systems (Iverson et al., 2009, pg. 181). In the central nervous system (CNS), both D_1 and D_2 are important in drug abuse, as both are implicated in the reinforcing effects of cocaine (Self & Stein, 1992). D_1 receptors, when activated, are stimulatory and therefore promote adenylyl cyclase activity. D_2 receptors, when stimulated, are inhibitory and inhibit adenylyl cyclase activity (Iverson et. al., 2009). This competing system of D_1 stimulation and D_2 inhibition may be modulatory in the presence of increased dopamine in the CNS, as D_1 functions to increase DA extracellular concentration and D_2 functions to decrease DA extracellularly (Iverson et al. 2009). Cocaine exposure has been found to decrease D_2 receptors in the medial prefrontal cortex (Briand et al., 2008). This reduction of D_2 receptors may decrease functioning of the regulatory system by stimulating the D_1 receptors in the NAc (Furst et al., 2013). The combination of the competing dopamine receptors may be the cause of the euphoric effects experienced after cocaine administration (Briand et al., 2008; Furst et al., 2013). The avian dopaminergic system has both similarities and differences to that of mammals. There are twice as many D_1 receptors as D_2 receptors in mammals, whereas, in the avian species, the number of D_2 receptors exceeds the number of D_1 receptors (Richfield, Young & Perry, 1987). Similar to mammalian findings, Japanese quail demonstrate increased locomotor activity (LA) after cocaine administration in CPP procedures and the D_2 receptor antagonist eticlopride attenuates the increase in LA activity (Post & Rose, 1976;

Levens & Akins, 2001). Also similar to mammalian research, D_2 antagonists do not attenuate CPP while D_1 antagonists block CPP (Cervo & Samanin, 1995; Akins, Levens, Prather, Cooper & Fritz, 2004). This similarity in the behavioral responses of the dopaminergic system between avian and mammalian species suggests that the dopamine system may be conserved across species.

Serotonin. At least 8 subtypes of serotonin receptors (5HT) have been identified, $5HT_1$ and $5HT_2$ families being the most common (Iverson et al., 2009). In the brain, there are two main projections of serotonin neurons: a caudal set that projects down to the medulla and spinal cord, and a rostral set that projects toward the raphe nuclei. Cocaine works by increasing extracellular expression of 5-HT by blocking serotonin's reuptake receptor (SERT). The rewarding aspects of cocaine have been specifically linked to 5-HT_2, subtype 5-HT_2c (Cunningham et al., 2011). Research has demonstrated that stimulation of $5HT_2$c decreases the reinforcing effects of cocaine. The antagonist of $5HT_2$c increases the reinforcing effects of cocaine, evident by high rates of self-administration of cocaine in animal models (Cunningham et al., 2011; Fletcher et al., 2009; Shalev et al., 2002; Grottick, Fletcher & Higgins, 2000). This together supports that $5HT_2$c may be an influential serotonin receptor in the reinforcing effects of cocaine.

Similar to mammals, birds have nine 5-HT receptor subtypes (Stepinska, Kuwana & Olszanska, 2014). However, some species differences have been reported. Rodents show a concentration of 5-HT_3 in brain tissue, in contrast to human and avian brain tissue (Peroutka, 1988). Nevertheless, 5-HT's ability to mediate dopamine's effects have been identified in birds (Zendehdel et al., 2014). Mediation of hypophagic effects exhibited

from cocaine administration via the serotoninergic system, specifically 5-HT_2c receptors, have been reported to negatively regulate DA release in the NAc and VTA through DA neurons. Pigeons given various agonists and antagonists of the serotoninergic system prior to cocaine administration have demonstrated the role of the 5-HT's mediation of DA neurons (Johanson & Barrett, 1993). In one of these studies, a discriminating stimulus effect was achieved via cocaine and a 5-HT_1 antagonist partially blocked these effects, further demonstrating the role of 5-HT mediation. In mammalian research, the 5-HT_2 antagonist predicts an increase in rates of responding in self-administration of cocaine, which can be interpreted as a reduction of the reinforcing effects of cocaine (Fletcher et al., 2009; Grottick et al., 2000). 5-HT mediation has been demonstrated in both avian species and mammalian, suggesting the serotonergic system's mediation of DA may be conserved across species.

Norepinephrine. Norepinephrine (NE) is a catecholamine in the sympathetic nerves of the peripheral nervous system (Iverson et al., 2009). NE is found in the synaptic vesicles of the sympathetic nerve ending of both the peripheral nervous system (PNS) and the central nervous system (CNS). There are two known classes of NE receptors, α and β receptors that are divided into 4 subtypes: α1, α2, β1 and β2 (Iverson et al., 2009). All NE tracts in the CNS depart from the locus coeruleus extending to the cerebellum, hippocampus and cerebral cortex (Iverson et al., 2009). Cocaine administration results in the increased extracellular NE concentration by blocking NET (Mannangatti, Arapulisamy, Shippenberg, Ramamoorthy & Jayanthi, 2011). In a study done by Mannangatti et al., (2011), the prefrontal cortex (PFC) exhibited increased concentration

of NE after administration of cocaine. Cocaine's effect on the peripheral system, via increased NE, include high blood pressure, dry mouth, pupil dilation, retention of urine and increased breathing (Iverson et al., 2009). Specifically, norepinephrine does not seem to play a large role in the rewarding effects of cocaine and research suggests only minor peripheral system fluctuations in response to cocaine due to NET blockade (Wang et al., 2013).

In the Japanese quail, NE is found in the highest concentration in the hypothalamus and the preoptic medial nucleus (POM) relative to other brain regions (Ottinger & Bathazart, 1987; Balthazart & Ball, 1989). Comparable to rodent research, there are gender differences in Japanese quail. First, within the POA, there is a higher concentration of NE in the female Japanese quail compared to male quail. Second, the NE turnover rate is gender specific such that the turnover increases with increased testosterone in males but not females (Balthazart, Libiulle & Sante, 1988; Ottinger & Bathazart, 1987; Rance, Wise, Selmanoff & Barraclough, 1981; Wise, Rance, Selmanoff & Barraclough, 1981; Hiemke, Bruder, Poetz & Ghraf, 1985). In addition NE increases with age for male Japanese quail, peaking at maturation (Duchala, Ottinger & Russek, 1984). It has also been demonstrated as influential, with 5-HT and DA showing a systematic decrease in response to NE depletion in Japanese quail (Balthazart et al., 1988). The combined roles of NE, DA and 5-HT may imply that NE is part of the global euphoric effects of cocaine (Pitts & Marwash, 1988). In quail, it has been demonstrated that cocaine blocks dopamine-β-hydroxylase (DBH), a precursor to both NE and DA synthesis in the LC in a dose dependent manner (Zhang et al., 1997; Purves, Augustin & Fitzpatrick

et.al., editors, 2001). This research may indicate that NE in the avian brain promotes DBH in the LC while cocaine inhibits DBH. Cocaine's inhibition of pre-curser DBH to DA in the LC may be evidence that the noradrenergic system may play a larger role in the effects of cocaine, than minor periphery effects in the Japanese quail.

Glutamate. In mammals glutamate is found as both an ionotropic and metabotropic glutamate receptor (Purves et.al. 2001). Glutamate ionotropic receptors are classified into three categories; NMDA, AMDA and kainate receptors. All categories are non-selective cation channels allowing the passage of Na+, K+ and to some extent Ca+ to pass through the membrane. Ionotropic glutamate receptors produce excitatory post-synaptic responses to activation unlike metabotropic glutamate receptors that can produce both. G protein linked second messenger systems classified as metabotropic glutamate receptors (mGluR) indirectly modulate post synaptic ion channels causing either an increase or decrease in post synaptic transmission (Iverson et al., 2009; Purves et al., 2001). MGluRs are responsible for regulation of ion channels and for various second messenger cascades in the central nervous system (CNS). MGluRs are the major modulatory second messenger of the CNS. There are three subtypes of mGluRs with various second messenger activation: subtype 1, mGluR1 and mGluR5; subtype 2, mGluR2 and mGluR3; and subtype 3, MGluR4, MGluR6 and MGluR7 (Iverson et al.,2009). Sensitization to cocaine has been linked to the activation of glutamate neurotransmitters, specifically to increased binding of AMPA receptors in the cerebral cortex and decreased binding in the PFC (Shaleve et al., 2002, Purves et al., 2001). Stimulation of glutamate receptors in the hippocampus, have been implicated in the reinstatement of cocaine

seeking behaviors (Vorel, Liu, Hayes, Spector & Gardner 2001; Blaha, Yang, Floresco, Barr & Phillips, 1997). Glutamate activation in the NAc and the ventral tegmental area (VTA) are implicated in the reinstatement of cocaine seeking in rodents (Shaleve et al., 2002).

Glutamate functions by controlling striatal DARPP-32 phosphorylation in mammal species (Nairn et al., 2004). DARPP-32 functions as an inhibitor of protein kinase A (PKA) while PKA functions to stimulate DARPP-32 through Thr_{34} (threonine). The inhibition of PKA through DARPP-32 is critical in the electrophysiological and behavioral responses of drugs of abuse. The role of DARPP-32 and the anticipation of reward has been published in mammalian literature (Svenningsson et. al., 2004). The use of DARPP-32 knock out (KO) mice have demonstrated the role of DARPP-32 in cocaine reward. Cocaine increases phosphorylation of DARPP-32 Thr_5 and decreases DARPP-32 Thr_{34}. This change in DARPP-32 phosphorylation results in behavioral changes: attenuation of both CPP and locomotor responses to cocaine.

Similar to mammals, aves receive glutamatergic input from the pallidum to the NAc and the ventral striatum (MSt) (Csillag, Balint, Adam & Zachar, 2008). Likewise, studies have identified glutamate in many of the same brain structures found in mammals (e.g. Perikarya, hippocampus, and nidopallium caudolaterale (NCL), mammal's prefrontal cortex equivalent). In the avian species, DARPP-32 has been found in high concentration along with glutamate in similar brain structures. The avian species has a high concentration of DARPP-32 in the MSt which is responsible for acquisition learning and anticipation of reward (Csillag et al., 2008). Cocaine may play a similar role with

DARPP-32 in the electrophysiological and behavioral responses of drugs of abuse in the avian species, suggesting that the glutamatergic system may be conserved across species.

Glucocorticoids. Glucocorticoids are steroid hormones that are released from the adrenal gland when cascade signals arrive from the anterior pituitary (Iverson et al., 2009). Release of glucocorticoids is self-regulating in that as levels of glucocorticoids increase, a built in feedback system shuts off the corticotrophin releasing factor (CRF). CRF is responsible for stimulation of the anterior pituitary that starts the release of signals to the adrenal gland. The feedback system turns CRF off when the adrenal gland registers excess of glucocorticoids (Ivesron et al., 2009). In rodents, the predominant glucocorticoid is corticosterone and it is found to be released following the activation of the hypothalamic pituitary gland via the hypothalamic–pituitary–adrenal axis (HPA) (Raubenheimer, Young, Andrew & Seckle, 2006; Shaleve et al., 2002). Research has shown that cocaine activates the HPA axis and chronic cocaine exposure can potentiate a CORT response (Larson, Schrott, Bordone & Sparber, 2001). Attenuation of cocaine-induced conditioned place preference (CPP) has been demonstrated with the use of non-selective receptor antagonists of CRF. (Sarnyai, Shaham, & Heinrichs, 2001; Schultz et al., 1996). This suggests that cocaine stimulates the HPA axis, specifically through CRF, thus increasing extracellular corticosterone.

Similar to rodents, the avian's predominant glucocorticoid is corticosterone (Ellestad, Puckett & Porter, 2015). In both mammals and birds, there are two main intercellular receptors of glucocorticoids that are changed by early life stress: mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) (Zimmer & Spencer,

2014). MR receptors are responsible for the overall sensitivity of the HPA axis with a high affinity for glucocorticoids while GRs work as an inhibitor of the HPA axis in aves. The overall CORT concentration in aves consists of an inverse U pattern across their life spans (Elliot et al., 2014). Whereas humans show a life-long increase of CORT and rodents show a lifelong decrease, the avian species is born with a high concentration of CORT that dips lowest in reproductive years and increases as they age (Elliot, et al., 2014). Prenatal CORT exposure in mammals has been shown to increase GR's and reduce MR's consequently, increasing the efficiency of the HPA axis. However, prenatal exposure to CORT in aves is mitigated by the presence of the egg that allows the offspring to be unaffected by the mother's potential CORT increases. Therefore, aves are less likely to succumb to changes from prenatal stressors. Japanese quail's HPA axis is protected from prenatal stressors that can alter their HPA axis sensitivity. This may make them of additional benefit to the study of cocaine effects in relation to stress.

Incentive Salience Theory

Incentive salience occurs when a subject attributes motivational properties to an otherwise neutral cue (Morrow, Maren & Robinson, 2011). The theory of incentive salience attempts to explain why those that attribute incentive salience to cues of reward may be more likely to relapse (Flagel, Watson, Robinson & Akil, 2007; Flagel, Akil & Robinson, 2009). When a conditioned stimulus (CS), is paired with an unconditioned stimulus (US), the CS may later elicit both a response to the reward as well as become an incentive stimulus (Pavlov, 1927, Meyer et al, 2012a). An incentive stimulus attracts attention, reinstates reward seeking and promotes acquisition of new behavior (Meyer et

al., 2012b, pg. 999). For a CS to acquire incentive salience, it needs to be attention-grabbing, desirable, and instigate reward seeking behavior (Di-Ciano, Cardinal, Cowell, Little & Everitt, 2001; Meyer et al., 2012a). When a CS has all of these traits, it can transform from a *predictive* cue to a "wanted" and sought after incentive stimulus (Saunders, et. al., 2013; Meyer et al., 2012b). The motivational property the cue takes on is established once the cue is no longer *predictive* and is subsequently *wanted*.

The attribution of incentive salience to cues is the foundation of cue-induced relapse. In the simplest form, an addict may begin to take drugs for the rewarding effects. When drug is paired with cues repeatedly, the cues may become a conditioned stimulus (CS). When the cues become predictive of drug, they may also acquire incentive salience and may instigate drug seeking and drug taking behavior (i.e. relapse) (Saunders et al., 2013; Flagel et al., 2009; Meyer et al., 2012a).

In the animal literature, sign and goal tracking are the behavior phenomenon that describes the individual differences in incentive salience attribution (Flagel et al.; 2009). A sign tracker (ST) spends the majority of the time engaging with the CS while a goal tracker (GT) spends the majority of the time with the US (Lovic, Saunders, Yager & Robinson, 2011). STs have shown cocaine-induced CPP to discrete cues while GTs have not (Meyer et al., 2012b). Together, this may mean that subjects with high incentive salience (STs) may be more vulnerable to cues previously paired with drugs.

Behavioral Procedures

Autoshaping**.** Research on incentive salience has been studied in animal models, resulting in both sign and goal tracking behaviors (Beckman & Bardo, 2012; Flagel et al.,

2009; Meyer et al., 2012). In rodents, a subject is presented with a lever (CS) paired with a food pellet (reward) (Beckman et al., 2012). After repeated pairings of the CS and reward, the lever becomes conditioned and may evoke motivation on its own. Once the CS has become conditioned, it may elicit behaviors similar to those predictive to the reward, i.e., licking, biting and sniffing (Beckman et al., 2012; Saunders et al., 2013).

Sign and goal tracking behaviors have also been identified in avian species (Burns & Domjan, 1996, 2000, 2001). In Japanese quail, a subject is presented with a CS (e.g. light, block) at one end of a chamber, paired with a female conspecific (US) at the opposite end of a chamber (Burns & Domjan, 1996, 2000, 2001). After repeated pairings of the CS and US, the CS becomes conditioned and may elicit approach to the CS (sign tracking behavior) or US (goal tracking behavior).

Conditioned Place Preference. Conditioned Place Preference (CPP) is a paradigm used to measure the learned association between a context and a reward. In CPP, a reward (i.e. drug) is paired with a context (i.e. color). In a 3 chamber apparatus, one context is paired with reward and another context with the absence of reward (Tzschentke, 1998). In mammals, most traditional CPP studies utilize shade (i.e. black and white) as a context. A rodent is presented with one shade (CS; black or white) paired with a drug (US) and another shade (CS) is paired with saline. After several pairings of the context and the drug, CPP is evident as a preference for the context that was paired with drug (Bardo & Bevins, 2000). A similar procedure to the one used with rodents is also used with aves. However, in these studies, contextual cues such as colored stripes and solid colored walls are used (Akins et al., 2004; Bolin, Cornett, Barnes, Gill & Akins, 2012).

Benefits of Using an Avian Model

Studies utilizing Japanese quail may offer several benefits over rodent models. The quail biology and behavior have been well established (see Mills, Crawford, Domjan & Faure, 1997 for Review). Most importantly, the dominant sense of quail is vision, unlike rodents that rely primarily on olfaction (Crombag, Badiani, Maren & Robinson, 2000; Whittow, 2000). Quail also have a visual acuity that is similar to that of humans. This may be important for incentive salience studies because it may allow them to discriminate differences between visual cues that are similar to each other (Tzschentke, 1998; Crombag et. al., 2000).

The use of Japanese quail as an incentive salience model is new. Currently there are no documented studies of incentive salience and drug abuse using Japanese quail. The rewarding effects of cocaine have been demonstrated in Japanese quail (Levens & Akins, 2001, 2004; Geary & Akins, 2007; Awaya & Watanabe, 2003). CPP, and sensitization have been used to study cocaine's effects and are well established in male Japanese quail (2001; Akins et al., 2004; Akins & Geary, 2008). Together, these findings suggest that Japanese quail might be of benefit to the study of incentive salience and drug abuse.

Chapter 2: Experiment 1

The theory of incentive salience postulates that cues associated with rewards may bias attention towards those cues and motivate reward seeking (Morrow et al., 2011; Robinson & Berridge, 1993). When cues that come to elicit approach through conditioning "become attractive" and motivate actions, they are said to have acquired incentive salience (Meyer et al., 2012; Saunders & Robinson, 2013; Robinson & Berridge, 2008). There are individual differences in the propensity to attribute incentive salience. For example, when a localizable cue (conditioned stimulus, i.e. CS) becomes associated with the receipt of food reward, for some rats ('sign trackers', STs), the cue itself becomes attractive, eliciting approach and engagement with it (Hearst & Jenkins, 1974). For these rats, the CS also serves as a potent conditioned reinforcer (i.e., STs will work to get it; Flagel, Akil & Robinson, 2009; Robinson & Flagel, 2009). For other rats ('goal trackers', GTs), the cue is equally predictive of reward (i.e., it serves as an effective CS), but they instead learn to approach the location of reward delivery, and for these rats the CS is relatively ineffective as a conditioned reinforcer (Robinson & Flagel, 2009; Yager & Robinson, 2010).

Identification of STs and GTs, is important in drug addiction research because sign tracking has been linked to drug-abuse behavior (Beckmann, Marusich, Gibson & Bardo, 2011). For example in rodents, sign trackers that attributed higher incentive salience to a food cue were later more likely to self-administer cocaine at a higher frequency than goal trackers that did not attribute incentive salience to a food cue (Beckmann et al., 2011). Other studies have demonstrated that sign trackers showed greater cocaine sensitization

(Flagel et al., 2007) and more robust rates of reinstatement to drug-paired cues than goal trackers (Saunders & Robinson, 2010).

The rewarding properties of cocaine have been investigated in animals that attribute incentive salience to cues using CPP. In a CPP study, Meyer et al., (2012) paired cocaine with one floor type (grid or holes) and saline with another. STs showed a place preference for the textured floor that was paired with cocaine whereas GTs did not. Similar studies have used other discrete cues, such as lights, levers and wood blocks, and found STs were more sensitive to discrete cues than GTs (Krank et al., 2008; Beckman et al., 2012; Doremus-Fitwater & Spear, 2011; Burns et al.; 2000). In sum, these studies establish that sign trackers may be more prone to attribute incentive salience to discrete cues previously paired with reward in relation to goal trackers. Therefore, to extend Meyers et al., (2012) research, the current experiment will use a discrete visual cue in a cocaine CPP paradigm.

Japanese quail are particularly sensitive to both colors and discrete local cues (Akins et al., 2004, Awaya & Watanabe, 2003). Colored walls have been employed as a CS for quail (Akins et al., 2004; Mace, Kraemer & Akins, 1997; Levens & Akins, 2001), as well as a variety of discrete cues (Burns & Domjan, 1996, 2000, 2001; Awaya & Watanabe, 2003). Domjan and colleagues, have used lights, wood blocks and taxidermic models as CS's in sexual conditioned approach studies with male Japanese quail (e.g. Burns & Domjan, 1996, 2000, 2001; Domjan et al., 1986, 1988). In these studies, male quail learned to approach discrete visual cues when they are followed by copulation with a female. In a study done by Awaya et al., (2003), a drug was paired with a specific quail

while saline was paired with another quail. In this study, the quail displayed individual preference for the quail that was paired with the drug. Therefore, Japanese quail could be of additional benefit to the study of approach behavior to discrete visual cues.

Previous rodent literature has shown the relationship of STs and GTs to drug seeking behavior with the use of CPP, specifically that STs acquired CPP to contextual cues and GTs did not (e.g. Meyer et al., 2012). To extend the research of Meyer et al., (2012), it was examined whether STs (subjects that attribute incentive salience to a cue) would show cocaine conditioned place preference to a discrete cue. The procedures used to identify individual differences in sign and goal tracking behavior were similar to those used with rodents (e.g. Meyer et al., 2012), except a discrete visual cue (colored light) was used as a conditioned stimulus. Once sign and goal trackers were identified and classified, the rewarding properties of cocaine were examined using a CPP procedure that utilized discrete cues rather than contextual cues. It was predicted that previously identified STs would exhibit a more robust cocaine CPP to a chamber containing a discrete cue compared to GTs.

Methods

Subjects and Housing

Forty-eight (N = 48) adult (6-7 months old) male Japanese quail *(Coturnix japonica*) supplied as eggs (from Kennewick, WA) were hatched at the University of Kentucky, and were experimentally and drug naive. One subject expired before completion of the experiment and was removed from analysis. The quail were maintained in mixed sex groups until approximately 4–5 weeks of age, then housed in

individual wire-mesh cages (GQF Manufacturing, Savannah, GA) and placed on a 16:8 hr light/dark cycle with food and water available *ad libitum*. Male quail were selected based on a pretest for sexual behavior. A female quail was placed in the home cage of each male for 5 min during a pretest. Only males that successfully copulated within 5 min were used in the experiment (Schein et al., 1972). Twenty-four (n = 24) sexually mature, age-matched female quail were used as copulation partners. All animal care and experimental procedures followed the guidelines of the Institutional Animal Care and Use Committee at the University of Kentucky.

Apparatus

Autoshaping. Autoshaping was conducted in chambers measuring 121.92 cm (long) × 60.96 cm (wide) × 30.48 cm (deep) (see Figure 1). The chambers had white walls and white floors. A side cage (30.48 cm wide × 30.48 cm deep × 30.48 cm tall) was attached to one end of the apparatus and was used to house the female bird during autoshaping. At the other end of the apparatus was a 0.015 W white LED light (the conditioned stimulus, CS) approximately 15.24 cm above the floor. An automated window connecting the male's test chamber to the female's side cage allowed male quail visual access to the female (US) when the window was open. The CS zone was a 30.48 cm x 60.96 cm area in front of the CS (light). At the opposite end of the chamber was a US zone (30.48 cm x 60.96 cm) in front of the US (female). The area in between the two zones measured (60.96 cm x 60.96 cm). The size of the chambers and the distance between the CS and US was selected based on previous sign tracking research with male Japanese quail (Burns & Domjan, 2001).

Conditioned Place Preference. Conditioned place preference was conducted in eight, three-compartment CPP chambers measuring approximately 68 cm long x 21 cm wide x 21 cm deep (ENV-013; MED Associates Inc., St. Albans, VT). The two outermost chambers (28.6 cm long x 21.2 cm wide x 21.2 cm deep) had white walls and were equipped with either a 12 V red or a 12 V green LED bulb. Each outermost chamber of the apparatus was equipped with six photo beams approximately 6.4 cm apart and 3.2 cm from the floor. The smaller central chamber (10.8 cm long x 21.2 cm wide x 21.2 cm deep) had gray walls and three photo beams also approximately 6.4 cm apart and 3.2 cm from the floor. The floors of each outer chamber were covered with wire mesh, the middle chamber with plastic flooring. A discrete red or green light was presented against a white background, at the end of each outermost chamber. Light color presentation was counterbalanced across groups (STs, GTs). White noise was used throughout each phase of the experiment to attenuate extraneous noise.

Drugs

Cocaine hydrochloride (National Institute on Drug Abuse; Bethesda, MD) was dissolved in physiological saline (0.9%) and injected intraperitoneally (ip) at a volume of 1-ml/kg body weight for a dose of 10 mg/kg. As a control, physiological saline (0.9%) was injected ip at a volume of 0.1 ml regardless of body weight.

Experiment 2A, Autoshaping

Procedures

Habituation. Subjects were placed in the center of the apparatus and allowed to freely explore the entire apparatus for 30 min a day for 2 days.

Autoshaping. During each autoshaping trial, male quail were placed into the center of the test chamber for a 90 sec variable interval (VI). Following the 90 sec VI, the CS light was illuminated for 8 sec and was followed by opening the window to the female's side cage. Males had visual access to the female for 8 sec. Previous studies with male quail have shown that visual access to female quail is sufficient to facilitate social proximity behavior that is persistent (Domjan et al., 1986; Domjan, O'Vary & Greene, 1988). Therefore, visual access to a female served as the US (goal) in the current study. Twenty five trials (1 session) were conducted per day for 5 days (sessions) for a total of 125 trials. Male quail were given 5 min to copulate with a female on day 1 and day 4 in their home cages (Schein, Diamond & Carter, 1972).

A difference score was used to identify the propensity of animals to sign and goal track. The difference score was calculated as: time (sec) spent in the CS zone minus time (sec) spent in the US zone during the CS presentation. The 125 difference scores were averaged for each subject, using SPSS version 21 software (SPSS Inc., Chicago, IL, USA). Based on the mean difference score, subjects were rank ordered. Similar to rodent models ITs were not used in subsequent testing (e.g. Yager & Robinson, 2010; Meyer et al., 2012).

Experiment: 2B, Conditioned Place Preference

Procedures

Thirty drug naive Japanese male quail, classified as GTs and STs via autoshaping and a one third rank order split, were used in the experiment. A biased CPP design was used in which drug was paired with the initially non preferred compartment (Nomikos &

Spyaki, 1988). CPP has been established previously using a biased design with 10 mg/kg cocaine in male quail (Akins et al., 2004; Levens & Akins, 2001) and the current procedures were similar to those studies. White noise was present throughout all phases of the experiment to attenuate extraneous noise.

Habituation**.** Subjects were habituated for 30 min per day. They were confined to alternating ends of the chamber every other day, for a total of 2 days in each side.

Pre-Test**.** The day after habituation, a place preference pre-test was conducted prior to conditioning to determine subjects' initial preference. During the preference test, subjects were allowed free access to the entire apparatus and could sample all three chambers. The preference test was conducted for 15 min. A place preference was determined as spending more time in one end chamber (e.g. with green light) as opposed to the other (e.g. with red light). Subjects not spending more time in one or the other end chamber (<50%) were randomly assigned a least preferred chamber. (Note that quail were given the preference test in a drug free state). The preference tests were recorded on Med PC software (ENV-013; MED Associates Inc., St. Albans, VT).

Conditioning Trials. During each conditioning trial, subjects received their assigned treatment (i.e. 10 mg/kg cocaine or saline) and were then confined to one end of the chamber for 30 min. On odd days, half the subjects were injected with 10 mg/kg cocaine and confined to one end of the chamber. The other half received saline and were confined to the other end chamber. On even days, all subjects received saline and were confined to the opposite end chamber. Photo beam breaks were recorded in the end chambers at 5 min bins using Med PC software (ENV-013; MED Associates Inc., St. Albans,

VT). The total number of beam breaks in the 30 min drug conditioning trials was used as an index of locomotor activity.

Post-Tests. Following conditioning, quail were given a place preference post-test after 8 alternating trials of saline and cocaine (Post-Test 1) and a second post-test after 8 additional alternating trials (Post-Test 2). Subjects were allowed free-access in a drug-free state to the entire CPP apparatus for 15 min and time spent in each chamber was recorded.

Statistical Analysis

Sign and Goal Tracking

To determine the acquisition of sign and goal tracking behavior across trials, a repeated-measures ANOVA with sessions as a repeated measure and sign or goal tracking behavior as a between-subject factor was conducted. Significant interactions were followed by independent one-way ANOVA's to further probe group x session interactions. Statistical significance was set at the $p < 0.05$ level.

Cocaine CPP

To determine whether place preference occurred in STs and GTs, a place preference was measured as a significant shift of time spent in the least preferred side where drug was conditioned from the pre to post test. Data were analyzed for STs and GTs separately, using a two factor repeated-measure ANOVA with group (cocaine vs saline) as the between subject factor and test (pre-test vs. post-test 1 vs. post-test2) as the repeated measure. Statistical significance was set at the $p < 0.05$ level.

Locomotor Activity

Beam breaks were analyzed during drug conditioning days using a repeated-measures ANOVA with treatment and group as the between subject factors and trial as the repeated measure.

Results

Figure 2 represents time spent (sec) sign tracking (panel A) and goal tracking (panel B) during the CS presentation across sessions for STs and GTs, as classified by the mean of their 125 difference scores. STs showed no change in sign tracking across sessions when compared to goal tracking, indicated by no significant behavior x trials interaction, $p > 0.05$. However, GTs increased their time spent goal tracking across sessions compared to sign tracking, indicated by a significant behavior x trial interaction, $F(4,112) = 2.886$, $p = 0.05$. In addition, an overall main effect of group was found, where STs spent significantly more time sign tracking than GTs overall, $F(1,28) = 19.491$, $p < 0.001$.

Figure 3 illustrates time spent (sec) in the drug-paired chamber for goal trackers (panel A) treated with 10 mg/kg cocaine (GTcoc) or saline (GTsal) and sign trackers (panel B) treated with 10 mg/kg cocaine (STcoc) or saline (STsal) during pretest, post-test 1, and post-test2. For GTs, there was a significant difference in the amount of time spent in the drug paired chamber across trials depending on treatment received. This was evident as a significant treatment x trial interaction, $F(2, 26) = 3.550$, $p < 0.05$. Further analysis revealed that GTcoc significantly increased time spent in the drug paired chamber from the pre- test to post-test1 ($F(1, 13) = 4.695$ $p < 0.05$). GTcoc decreased time spent in the

drug paired chamber from post-test 1 to post-test 2, F (1, 13) =6.864, $p < 0.05$. No significant treatment x trial interaction was found for STs, F (2, 26) =.172, $p > 0.05$.

Figure 4 illustrates the frequency of photo beam breaks for GTs (panel A) treated with 10 mg/kg cocaine (GTcoc) and saline (GTsal) and STs (panel B) treated with 10 mg/kg cocaine (STcoc) and saline (STsal) across cocaine conditioning trials (odd days). Subjects differed based on group and treatment across trials resulting in a three way interaction of treatment x group x trial, F (7,182) 2.383, $p < 0.05$. Independent ANOVA's for STs and GTs indicated a treatment x trial interaction for STs, F (7, 91) 3.157, $p < 0.01$, but not GTs, F(7,91)= 1.235, $p > 0.05$. Further probing of the interaction for STs indicated that STcoc (M=3803.464, SEM= 411.65) had increased activity compared to STsal (M= 3680.563, SEM= 385.069), F (7, 91) 3.157, $p < .05$.

Discussion

The present study asked whether STs (subjects that attributed incentive salience to a cue) would also demonstrate cocaine CPP to a discrete cue. STs and GTs were classified using a rank order split based on their mean difference score. STs spent significantly more time sign tracking compared to GTs. Likewise, GTs spent more time goal tracking, compared to STs. The CPP results showed that GTs acquired CPP to a discrete cue but STs did not. However, STs showed enhanced locomotor activity to cocaine during conditioning compared to GTs.

Previous rodent literature has shown that STs acquired CPP and GTs did not (e.g. Meyer et al., 2012). The results of Meyer's study reported that STs that attributed incentive salience to a cue showed a CPP to a contextual cue paired with cocaine. Based

on Meyer's results, it was hypothesized that STs would also show cocaine CPP to discrete visual cues, and GTs would not. Following screening for STs and GTs, the current research employed a discrete visual cue (i.e. colored light) and paired one color (red or green) with cocaine and another color with saline. Contrary to our hypothesis, STs did not acquire cocaine CPP to a discrete visual cue while GTs did.

One possible explanation for STs not acquiring CPP in the current study is that STs did not learn the relationship of the drug cue and cocaine and may have experienced latent inhibition. Latent inhibition is the learning phenomenon in which a CS is presented without the US and may therefore delay conditioning of the CS-US relationship when they are later paired. Bougher and colleagues (2003) studied latent inhibition in a rodent model and found STs demonstrate latent inhibition with only one pre-exposure to a cue (i.e., light). In the current work, the subjects had 2 exposures of the cue while the light was off (habituation) and 3 additional experiences with the cue while it was on (preference tests). Consequently, it is possible that STs were unable to learn the relationship of the light cue with cocaine as a result of latent inhibition.

A more likely explanation is that the use of the one third rank order split to classify sign and goal trackers may have misclassified STs. In the current experiment, a rank order split of the mean difference score was used to classify STs and GTs similar to previous rodent literature (e.g. Flagel, et al. 2007). This relative criterion may be prone to misclassification of STs and GTs as it relies on the behavior of the other subjects and not an absolute criterion. The rank order split is a relative approach and a pre-specified number of STs and GTs are guaranteed. One problem with this approach is that if none of

the subjects are exhibiting sign tracking behavior, they will still be ranked in the top third and classified as STs. Similarly, if none of the subjects are exhibiting goal tracking behavior, one third will still be classified as GTs. Inaccurate classification of STs and GTs introduces “noise” into the data and attenuates effect sizes. It is therefore possible that subjects were misclassified with this approach in the current experiment.

Figure 1

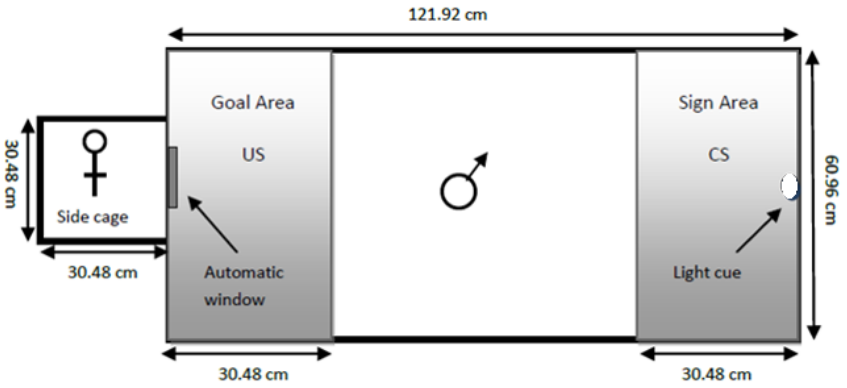

Figure 1: Diagram of the apparatus used in experiment. The area marked off in front of the window was the scoring area for time spent in the US zone. The area marked off in front of the light was the scoring area for time spent in the CS zone.

Figure 2

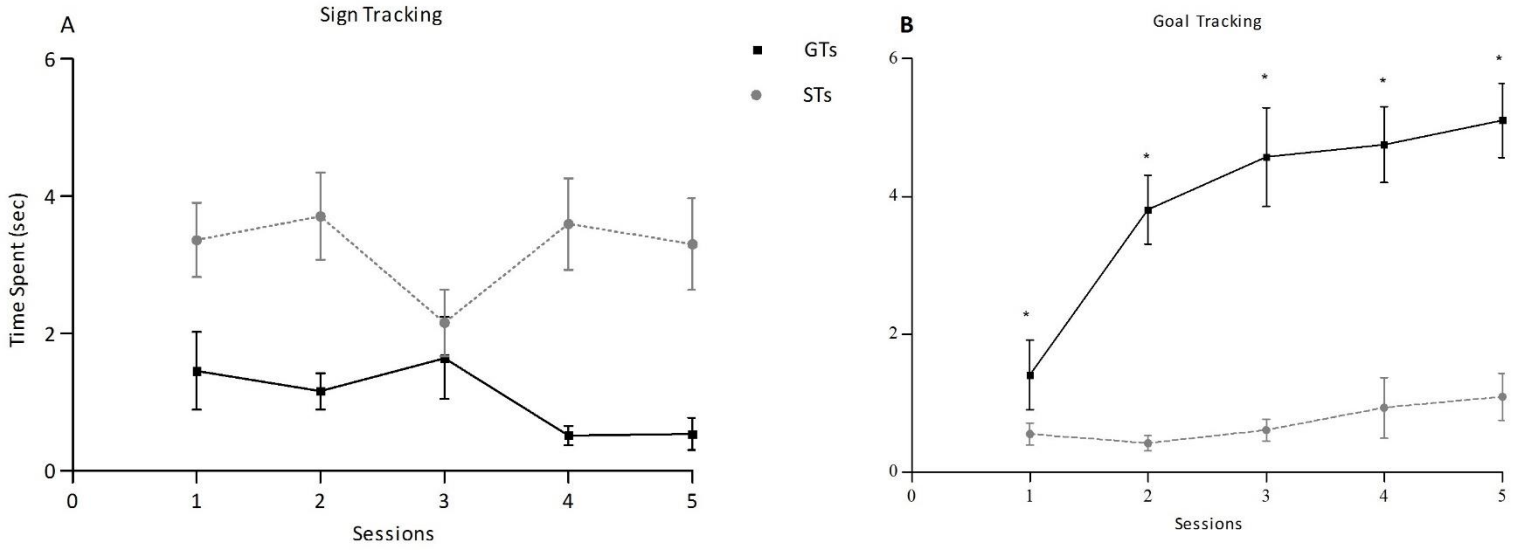

Figure 2. Time spent (sec) sign tracking (Panel A) and goal tracking (Panel B) during the CS presentation across 5 sessions (25 trials a session) for STs and GTs identified with the difference score. * = GTs > STs, $p < 0.05$.

Figure 3

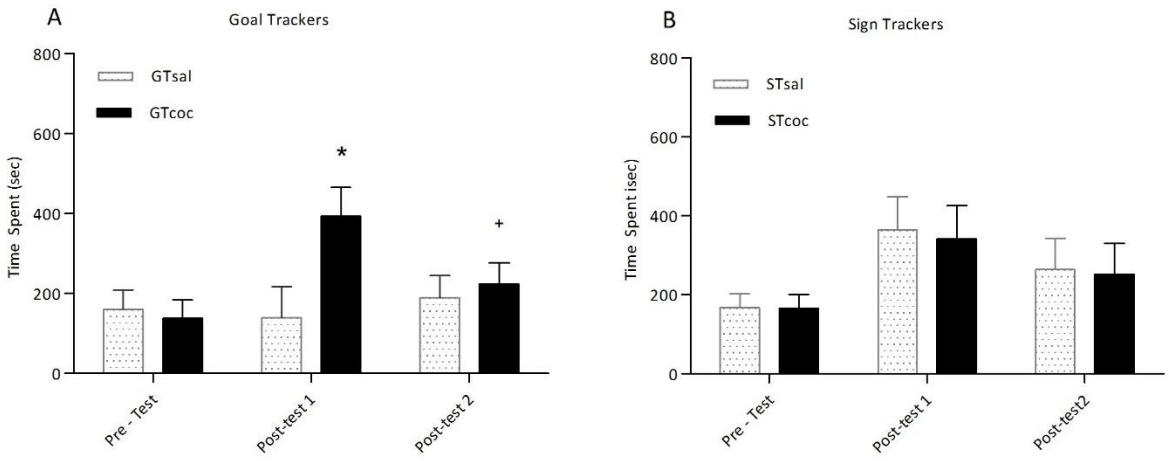

Figure 3. Time spent (sec) in the drug paired chamber for goal trackers (Panel A) that received 10 mg/kg cocaine (GTcoc) and saline (GTsal) and sign trackers (Panel B) that received 10 mg/kg cocaine (STcoc) and saline (STsal) for Pre-Test, Post-test 1 and Post-test 2. * indicates a significant difference of Pre-Test and Post-test 1 for GTcoc $p < 0.05$, + indicates a significant difference between Post-test 1 and Post-test 2 for GTcoc $p < .05$.

Figure 4

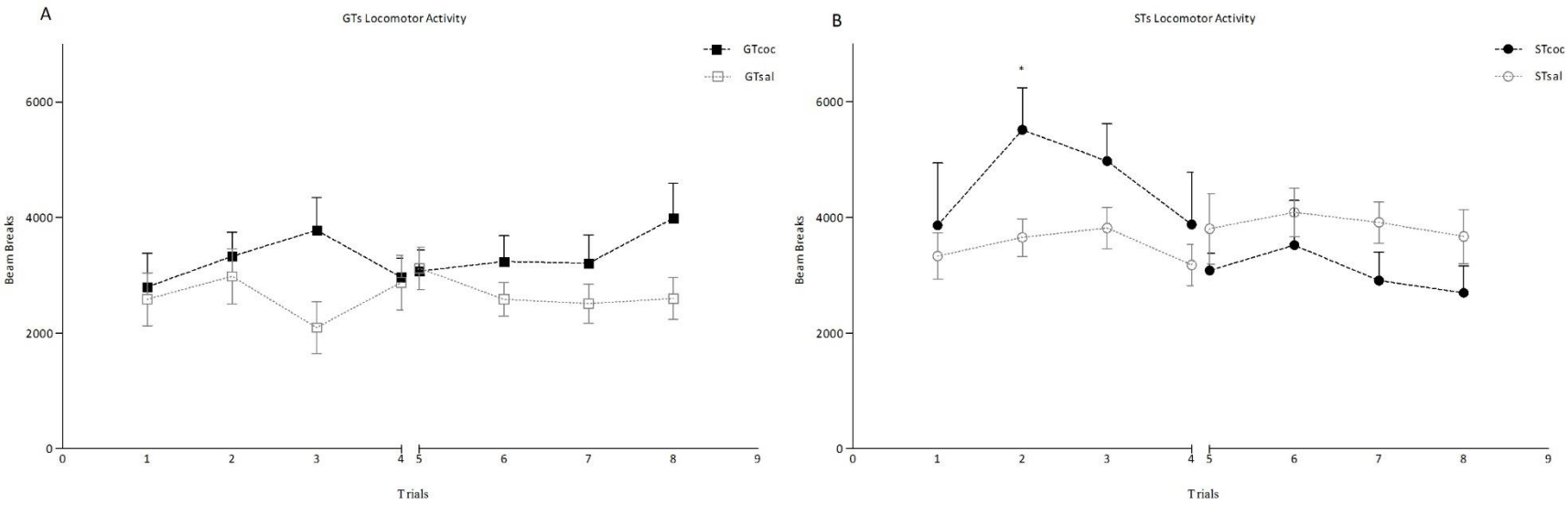

Figure 4. Mean (± SEM) beam breaks for GTs (panel A) that received 10 mg/kg cocaine (GTcoc) or saline (GTsal) and STs (panel B) that received 10 mg/kg cocaine (STcoc) and saline (STsal) across conditioning days. * indicates STcoc > than STsal, $p < .05$.

Chapter 3: Experiment 2

The previous experiment investigated whether sign trackers (subjects that attributed incentive salience to a cue) would demonstrate cocaine CPP to a discrete cue. A rank order split (a relative criterion) was used to identify sign trackers (STs) and goal trackers (GTs). Results of that research showed that STs did not acquire CPP to a discrete visual cue. However, it is possible that the procedure used to categorize STs and GTs, misclassified the subjects. The goal of the current experiment was to investigate the validity of the rank order split when classifying STs and GTs as opposed to an absolute criterion (t-test identification). It was predicted that the t-test would yield a more accurate identification of sign and goal trackers than the rank order split.

Various methods have been used to identify sign and goal trackers. One method is the difference or elevation score that consists of subtracting time spent near the CS during the pre-CS time (prior to the presentation of the CS) from the CS time (Costa & Boakes, 2009; Krank, O'Neill, Squarey & Jacob 2008; Palmatier et al., 2013). Another method utilized is the probability score that divides time spent near the CS by time spent near the US (Chang & Holland 2013; Tomie, Lincks, Nadarajah, Pohorecky & Yu, 2012). These methods subsequently classify STs and GTs using a 1/3rd rank order split method — dividing the sample into thirds based on the response used to identify them as used in the previous research. The top third are classified as sign trackers and bottom third as goal trackers, while the middle (intermediates) are not typically included in further analyses. Thus, the rank order split uses a "relative" criterion in that it classifies

individuals based on how their behavior compares to other animals in the sample. With a small sample size, this could potentially result in misclassifications of individuals.

One method that does not rely on the rank order split is the Pavlovian conditioned approach (PCA) index. The PCA index is calculated using multiple measures relating to the frequency, probability, and latency of behaviors directed toward a CS lever (sign tracking) and toward a US food cup (goal tracking; originally described in Meyer et al., 2012). Using this index, sign trackers are those with a score of greater than 0.5, goal trackers are those with a score of less than -0.5, and intermediates are those in between. The PCA index utilizes an "absolute" criterion based on a composite score and it therefore more accurately and less arbitrarily identifies sign and goal trackers than the rank order split method.

However, the PCA index also has some limitations. First, it allows for cross-comparison of studies but only those studies that utilize rodents and collect the same measures of sign and goal tracking. While the measures collected are comprehensive, other measures of sign and goal tracking behavior that are not specific to rodents might also contribute to the field. The PCA index does not allow for other measures of sign and goal tracking nor the use of other species that may not press a lever or eat out of a food cup. Second, use of the PCA index requires a relatively large sample size which may not be practical under most circumstances. Finally, use of the PCA index does not take into account trial by trial variability. This may be important in examining shifts in sign and goal tracking behavior across time.

One animal model that might be of additional value in studying sign and goal tracking behavior is an avian species, Japanese quail. Japanese quail are a visually-oriented species with color vision and high visual acuity (Mills et al., 1997). It is widely accepted that visual cues in the environment may become associated with drug taking and later, in the absence of drug, cause drug-seeking and ultimately relapse. Therefore, quail have served as a visual model in numerous studies on drugs of abuse (e.g. Bolin et al., 2014; Bolin & Akins, 2012; Rosine, Bolin & Akins, 2009). In addition to serving as a visual model in substance abuse studies, male quail exhibit a sexually conditioned approach response that has been well established (Domjan, Lyons, North & Bruell, 1986). In this paradigm, male quail receive a visual stimulus (CS) followed by copulation with a female quail. After several pairings of the stimulus with copulation (US), males learn to approach the visual stimulus that predicts the female. The conditioned approach behavior is similar to sign tracking except that the sign and goal are located in the same location. The first sign and goal tracking experiment was performed in pigeons with the sign and goal located at opposite ends of a chamber. The current experiment was modeled after this study (Hearst & Jenkins, 1974) and recent sign and goal tracking studies conducted in male Japanese quail (Burns & Domjan, 1996, 2000).

Experiment 2 proposes an alternative classification method to the rank order split and PCA index. While the research was conducted with Japanese quail, it is proposed that the use of the alternative classification of sign and goal trackers can be used with a wide variety of species that demonstrate distinct sign and goal tracking behavior. The method involves using one sample t-tests to determine whether mean difference scores are

significantly different from zero. A statistically significant positive t score would result in classification of subjects as sign trackers (STs). A statistically significant negative t score would result in classification as goal trackers (GTs). Subjects with difference scores that are not significantly different from 0 would be classified as intermediates (ITs). In this way, any number of subjects could be classified as STs or GTs and this classification would be based solely on the subject's behavior and not the behavior of the other subjects. The current study illustrated this approach and compared its accuracy to the traditional rank order split approach.

Methods

Subjects and Housing

Seventeen (N = 17) adult (6-7 months old) male Japanese quail *(Coturnix japonica*) that were supplied as eggs from Northwest Game birds (Kennewick, WA) were hatched and used as subjects. Quail were maintained in mixed sex groups until approximately 4 weeks of age, then housed in individual wire-mesh cages (supplied by GQF Manufacturing, Savannah, GA) and placed on a 16:8 hr light/dark cycle with food and water available *ad libitum*. Male quail were selected after successful copulation within 5 min. with a female (N=8, sexual mature, aged matched) in their home cage (Schein et al., 1972).

Apparatus

Autoshaping. A diagram of the apparatus used in the current experiment is shown in Figure 1 of Experiment 1. Autoshaping was conducted in chambers measuring 121.92 cm (long) × 60.96 cm (wide) × 30.48 cm (deep). The chambers had white walls and

white floors. A side cage (30.48 cm wide × 30.48 cm deep × 30.48 cm tall) was attached to one end of the apparatus and was used to house the female bird during conditioning. At the other end of the apparatus was a 0.015 W blue LED light (the conditioned stimulus, CS) approximately 15.24 cm above the floor. An automated window connecting the male's test chamber to the female's side cage allowed male quail visual access to the female (US) when the window was open. The CS zone was a 30.48 cm x 60.96 cm area in front of the CS (light). At the opposite end of the chamber was a US zone (30.48 cm x 60.96 cm) in front of the US (female window). The area in between the two zones measured (60.96 cm x 60.96 cm). The size of the chambers and the distance between the CS and US was selected based on previous sign tracking research with Japanese quail (Burns & Domjan, 2001).

Pavlovian conditioning

Habituation

All birds received 3 days of habituation in the apparatus. During habituation, male birds were placed in the test chamber and given 30 min of exposure, once daily for three days. The CS (light) and US (female) were not present during habituation.

Autoshaping

Following habituation, autoshaping trials were conducted. During each conditioning trial, male quail were placed into the center of the test chamber for a 90 sec variable interval (VI). Following the 90 sec VI, the CS light was illuminated for 10 sec and was followed by opening the window to the female's side cage. Previous studies with male quail have shown that visual access to female quail is sufficient to facilitate social

proximity behavior that is persistent (Domjan et al., 1986; Domjan et al, 1988). Therefore, visual access to a female served as the US (goal) in the current study. Five trials (1 session) were conducted per day for 25 days (sessions) for a total of 125 trials. Male quail were given 5 min to copulate with a female every 3rd day in their home cages (Schein et al., 1972).

It should be noted that after session 3, the CS light broke and was replaced with a 0.045 W light for trials 16-45 instead of a 0.015 W light. However, the differences in light W were unrelated to subject behavior based on a multilevel modeling analysis, B = -0.14, p = 0.75. Therefore, data from all trials were included in future analyses.

Statistical Analyses

Difference Score

A difference score was used to identify the propensity of animals to sign and goal track. The difference score was calculated as: time (sec) spent in the CS zone minus time (sec) spent in the US zone during the CS presentation.

T-Test Approach

Each subject's difference scores on the 125 trials were analyzed using a one-sample t-test with a test value of 0, using SPSS version 21 software (SPSS Inc., Chicago, IL, USA). Statistical significance was determined at $p < 0.05$. Those subjects with significantly positive difference scores (significantly more time in CS zone than US zone) were classified as STs. Subjects with significantly negative difference score (significantly more time in US zone than CS zone) were classified as GTs.

One third Rank Order Split

The 125 difference scores were averaged for each subject. Based on this mean difference score, subjects were rank ordered using a 1/3rd split. The 6 subjects with the highest mean difference scores were classified as STs, the 6 subjects with the lowest mean difference scores were classified as GTs, and the remaining subjects were classified as ITs.

Results

Prior to comparing the accuracy of the t-test approach with the rank order split method of classification, the propensity of sign and goal tracking behavior for STs, GTs, and ITs as classified using the t-test approach was analyzed across sessions. Figure 5 represents time spent (sec) sign tracking (Panel A) and goal tracking (Panel B) during the CS across sessions for STs, GTs, and ITs as classified by the t-test approach, presented in 25 trial sessions. For sign tracking behavior (Panel A), STs increased their time spent sign tracking across sessions compared to GTs and ITs. This was indicated by a significant group x trials interaction, $F(2,14) = 10.76$, $p = 0.001$. Posthoc analyses indicated that STs spent significantly more time sign tracking than GTs on session 3 and more than GTs and ITs on session 4. For goal tracking behavior (Panel B), there was no significant group x trials interaction, $F(8, 56) = 0.465$. However, upon closer examination of goal tracking behavior (Panel C), goal trackers increased time spent goal tracking across the first 5 trials compared to the amount of time they spent sign tracking, $F(1, 42) = 12.699$, $p = 0.001$. Subsequent posthoc analyses revealed that goal trackers spent more time goal tracking than sign tracking on trials 3, 4, and 5.

Table 1 illustrates the mean difference scores for individuals and classification of STs, GTs, and ITs based on either the use of a rank order split (column 2) or a statistically significant t-score (column 3). Using the rank order split, 6 STs, 6 GTs, and 5 ITs were classified. In contrast, using the t-scores, 4 subjects were classified as STs, 9 as GTs, and 4 as ITs. Comparison of columns 1 and 2 reveals the difference in classification between the t-test method and the rank order split method. Two subjects classified as STs by the rank order split did not exhibit sign tracking on average. This is illustrated by their negative mean difference scores. The t-test approach more accurately classified them as ITs. In addition, the rank order split method classified 3 subjects as ITs who spend spent significantly more time in the US zone than the CS zone. The t-test approach accurately classified these subjects as goal trackers.

Figure 6 shows mean difference scores for approach behavior across 25 sessions for quail classified as STs, GTs or ITs based on the rank order split classification (Panel A) and the t-test approach (Panel B). Separate repeated-measures ANOVAs revealed a significant main effect of group for both approaches (rank order split: F (2, 14) = 11.33, p = 0.001; t-test: (2, 14) = 11.98,p = 0.000). The rank order split post hoc analyses showed that STs (M = 3.36, SEM = 0.98) had a higher mean difference score than GTs (M = -1.508, SEM = 0.67). In the t-test approach, the post hoc analysis indicated that the mean difference score of STs (M = 1.34, SEM = 1.22) was significantly higher than that of GTs (M = -0.91, SEM = 0.28). Additionally, the mean difference score of ITs (M = -0.61 SEM = 0.23) was significantly lower than GTs (M = 0.91, SEM = 0.28). Thus, overall analyses resulted in similar outcomes of sign and goal trackers using each approach.

The repeated-measures ANOVAs also revealed a significant main effect of session for the t-test approach, $F(24, 720) = 2.08$, $p = 0.002$, but there was no significant main effect of session for the rank order split approach, $F(24,720) = 0.56$, $p = 0.6$. Further post hoc analyses for the t-test data found significant session differences at sessions 5, 6, 12, 17 and 19 ($p < 0.05$). Based on these analyses, it appears that use of the rank order split resulted in an artificial IT group, whereas use of the t-test approach revealed that the IT group was actually switching from sign tracking to goal tracking over time. It is important to note that this outcome is not a function of the species or procedures but rather a function of the method used to analyze the data.

Discussion

Attribution of incentive salience to drug cues is linked to relapse in drug taking (Saunders & Robinson, 2013; Saunders & Robinson, 2010). However, only some individuals display behavior that represents incentive salience attribution (Meyer, et al., 2012). Accurate identification of those individuals is critical for addiction research. The current study used a t-test approach of classifying subjects that engage in attribution of incentive salience (sign-trackers) and those that do not (goal-trackers and intermediates). This classification was based on subjects' time spent near a CS and US across trials. Rather than relying on a classification criterion that compares subjects to each other, use of the t-test approach classified subjects based on an absolute criterion such that subjects had to have spent significantly more time in the CS zone than the US zone to be classified as a ST, and significantly more time in the US zone than the CS zone to be

classified as a GT. The current study compared use of the t-test approach with the commonly used one third rank order split method.

Prior to examining the comparison of the rank order split to the t-test approach, the amount of time spent sign tracking and goal tracking during presentation of the CS was analyzed across conditioning sessions in birds who were classified as sign and goal trackers according to the t-test approach. The findings indicated that birds that were classified as STs spent significantly more time sign tracking across sessions than those classified as GTs and ITs. Thus, it was clear that the sign tracking behavior that STs demonstrated across sessions was due to learning. When a similar analysis was conducted for GTs, there was no significant interaction across sessions and thus, it appeared that the goal tracking response was not a result of learning. However, upon closer examination, GTs appeared to show an increase in goal tracking behavior across trials 1-5 compared to STs and ITs that did not. Thus, the goal tracking behavior exhibited by GTs was also a learned response but appeared to be learned very early in the sessions.

Using the t-test approach, we classified 4 STs, 9 GTs, and 4 ITs whereas use of the rank order split misclassified 5 subjects. Two subjects that did not exhibit sign tracking were misclassified as STs and 3 subjects that spent significantly more time goal tracking were misclassified as ITs. Misclassification of sign and/or goal trackers within a study is problematic because these classifications are used to determine future drug abuse vulnerability. Researchers typically screen for sign and goal trackers, classify them, and use them in their group assignment to study other drug-related phenomena. For example, if an animal is classified as a sign tracker when it is actually an intermediate, the

validity of subsequent tests and the interpretation of those findings might be questionable. Furthermore, using the t-test approach, it was shown that the ITs may be switching to goal tracking. This was not evident using the rank order split classification. The more accurate account for the behavior seen as 'switching' in the IT's is shown in figure 3B (t-test approach) and not figure 3A (rank order split classification). Therefore, the t-test approach allowed for a more accurate account of what the animals were doing.

It should be noted that t-tests have been used in previously published sign and goal tracking research (Flagel et al., 2008). Flagel, et al., (2008) first used the 1/3rd rank order split to classify sign and goal trackers. A t-test was then used to compare the groups that were previously classified using a relative criterion. Therefore, use of the t-test in that study was not for classifying subjects and is unlike the proposed t-test approach.

The t-test in the current research applied the use of all 125 trials, whereas the rodent literature has used only select trials in determining sign and goal tracking. Some rodent researchers include all trials (Flagel et al., 2008; Krank et al., 2008) while others include every 5th day (DiFeliceantonio & Berridge, 2012) as well the last day (Paolone, Angelakos, Meyer, Robinson & Sarter, 2013) or last 2 days (Lovic et al., 2011). The rationale for only including the last 1 or 2 days of testing in the analyses is that presumably stabilization of the behaviors has been achieved at that point (Lovic et al., 2011). The current research employed the use of all 125 trials to classify STs and GTs to capture the full behavioral repertoire across sessions and to preserve statistical power.

In the current study, we only classified 4 STs so it might appear that use of the t-test approach requires screening of large numbers of subjects to attain enough sign and goal trackers for subsequent testing. However, because the approach classifies subjects more accurately, this may result in larger effects sizes and therefore increased power, allowing for smaller numbers of subjects for subsequent testing.

The t-test approach can be easily adapted to other indices of sign and goal tracking. It can be used to analyze raw data such as time spent in CS zone (Burns & Domjan, 1996), elevation scores that include other behaviors such as dipper entries (Palmatier et al., 2012), and PCA data (Meyer et al., 2012). It can also be used to examine sign and goal tracking behavior that is not specific to rodents. The response measure of interest could simply be averaged across all trials for each subject and compared to a criterion value using the one sample t-test. In all cases, use of the t score should more accurately identify the subset of subjects hypothesized to be most vulnerable to drug addiction. This novel tool may be of value for use in future drug addiction studies.

Table 1

Subject	Classification X Rank Order Third Split	Classification X T Score	Total Mean Differences Score	T score (124)	SEM
307	ST	ST	4.17	9.83*	0.42
310	ST	ST	2.83	5.62*	0.54
309	ST	ST	1.07	2.86*	0.38
272	ST	ST	0.77	1.81*	0.42
299	ST	IT	0.28	1.47	0.19
304	ST	IT	-0.08	-0.14	0.60
302	IT	IT	-0.15	-0.46	0.33
293	IT	IT	-0.33	-1.25	0.26
308	IT	GT	-0.60	-2.17*	0.28
305	IT	GT	-0.74	-2.20*	0.34
300	IT	GT	-1.20	-4.83*	0.25
311	GT	GT	-1.59	-3.37*	0.47
271	GT	GT	-1.87	-5.37*	0.35
270	GT	GT	-2.45	-6.19*	0.40
303	GT	GT	-2.84	-8.70*	0.33
298	GT	GT	-3.19	-8.93*	0.36
301	GT	GT	-8.18	-23.91*	0.34

Table 1. Classification of STs, GTs, and Intermediates based on the 1/3 rank order split (column 2) and the T score (column 3). SEM = standard error of means; * $p < .05$.

Figure 5

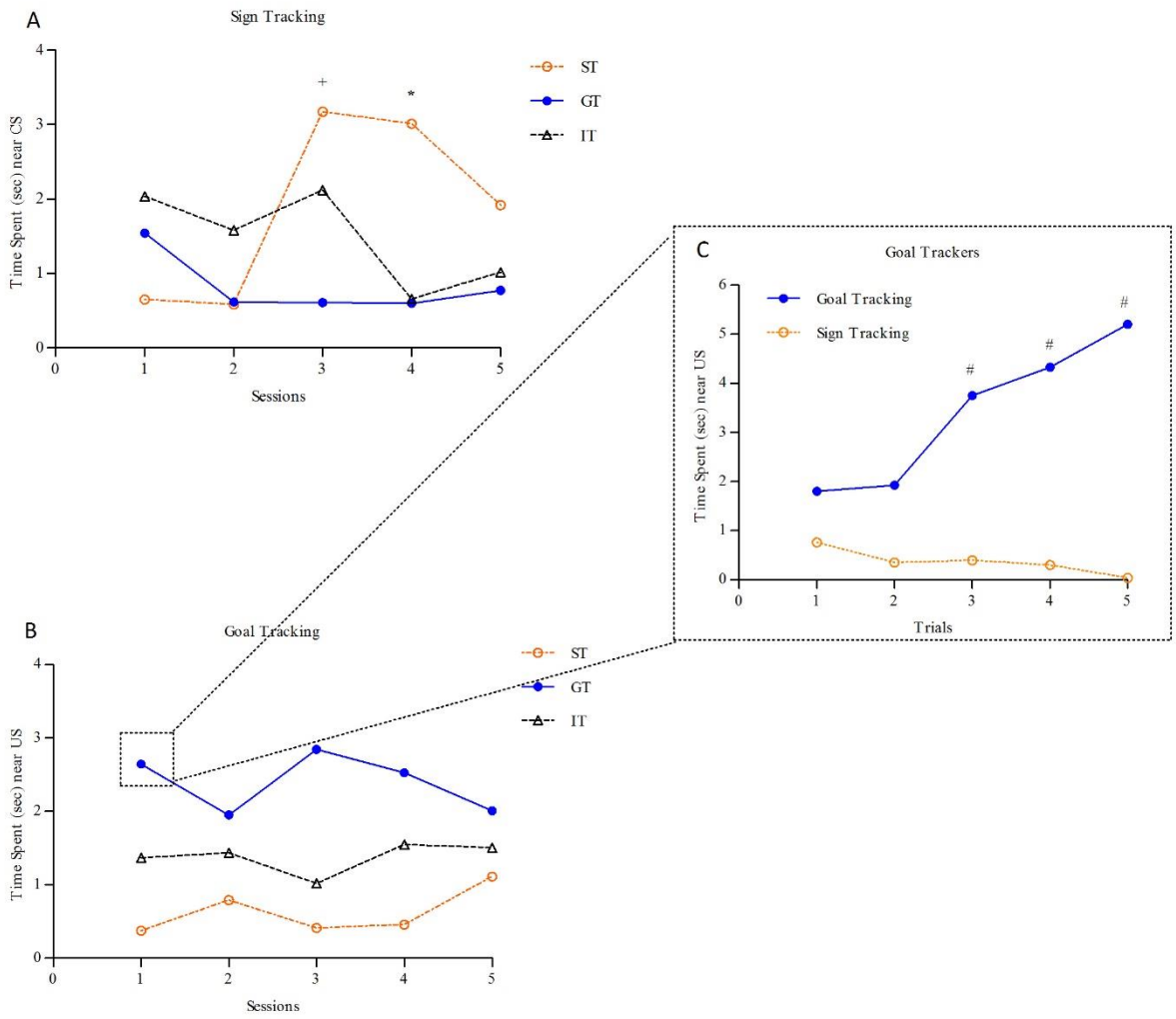

Figure 5. Time spent (sec) sign tracking (Panel A) and goal tracking (Panel B) during the CS presentation across 25-trial sessions for subjects classified as STs, GTs, and ITs using the t-test approach. Panel C represents time spent (sec) sign and goal tracking across the first 5 trials for subjects classified as goal trackers based on the t-test approach. + = STs > GTs; * = STs > GTs and ITs; # = Goal tracking > sign tracking

Figure 6

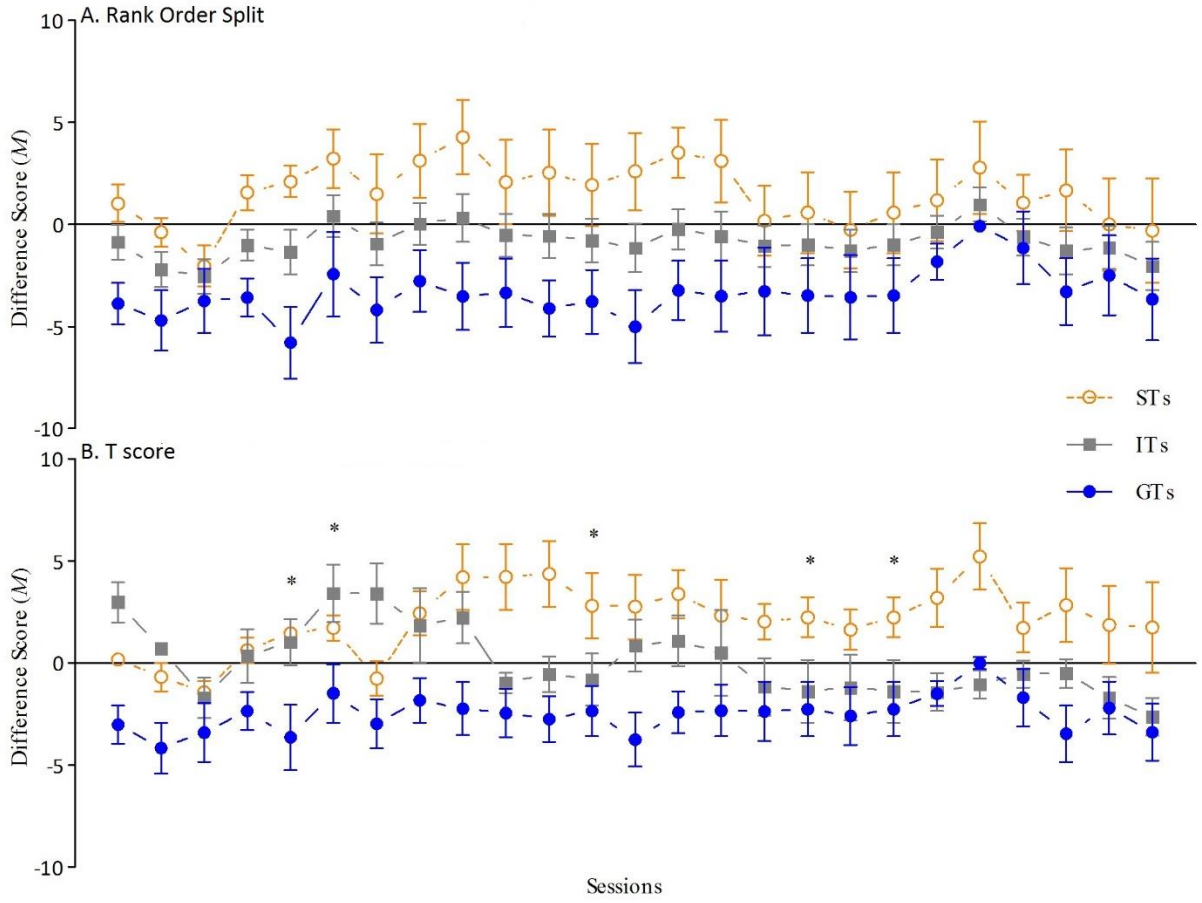

Figure 6. Mean difference scores of approach behavior across 25 sessions for STs, GTs and ITs based on the rank order split method (Panel A) and the T score approach (Panel B). * indicates significant session effects between STs and GTs, $p < .05$.

Chapter 4: General Discussion

The overall goal of the current work was to investigate whether STs that attribute incentive salience to a cue would acquire cocaine conditioned place preference (CPP) to a discrete cue. In Experiment 1, sign and goal trackers were given an autoshaping procedure and identified with a rank order split. Results showed that GTs acquired a cocaine CPP to a discrete cue but STs did not. A possible explanation for the findings of Experiment 1 might have been misclassification of the subjects. Therefore, Experiment 2 investigated the use of the rank order method used to identify STs and GTs in Experiment 1 and compared the rank order to an absolute criteria, a t-test classification. Experiment 2 found that misclassification of STs and GTs does occur with the rank order classification method and that the t-test classification is more accurate.

The findings in Experiment 1 were in contrast to previous rodent literature. Meyer et al. (2012) found that STs did acquire cocaine CPP and GTs did not. The most likely explanation for STs not acquiring cocaine CPP in the current study was the misclassification of STs by the use of the one third rank order split. In Experiment 1, the most common approach of identifying STs and GTs, a rank order of subject's behavior based on an index of sign tracking (a relative criterion; compared subjects behavior to each other) was used to classify subjects. The subject's time spent near the US was subtracted from the time spent near the CS while the CS was on, resulting in a difference score. The mean difference score was then rank ordered. Male quail with the highest third difference score were classified as STs, while the lowest third were classified as GTs

and the middle third were classified as ITs (Intermediates). Thus, the classification procedure was a relative approach and a pre-specified number of STs and GTs were selected. One problem with this approach is that if none of the subjects are exhibiting sign tracking behavior, they were still ranked in the top third and classified as STs. Similarly, if none of the subjects were exhibiting goal tracking behavior, one third were classified as GTs. Therefore, Experiment 2 sought to investigate this possible error in classification of STs and GTs.

Experiment 2 compared the t-test approach and the rank order split that was used in Experiment 1. The rank order split was found to have misclassified 30% of the subjects in Experiment 2 with both sign and goal trackers. Therefore, it is also possible that a similar percentage of misclassification would be expected in Experiment 1. Hypotheses that are contingent on identification of STs and GTs lose power and introduce 'noise' into the data, when using misclassified subjects. In Experiment 1 the research hypothesis that STs would acquire cocaine CPP to a discrete visual cue relied heavily on the classification of STs. The results of Experiment 2 indicated a possible explanation for why STs did not acquire cocaine CPP in Experiment 1, because they may have been misclassified. Had a more accurate classification method such as the t-test been used, the results of Experiment 1 may have been different.

Current literature is inundated with studies using rodent models to explain the relationship of cues that acquire incentive salience to drug abuse. Considering that cues that become associated with drug taking are often visual, a species with a primary sense of vision offers an alternative model for sign and goal tracking studies compared to the

traditional rodent model. The current sign and goal tracking model, utilized an avian species to investigate cocaine CPP with discrete visual cues. Experiment 2 demonstrated the likelihood that one method currently used, the rank order split, misclassifies STs and GTs. The same experiment demonstrated a more accurate method to classify the same behaviors, the t-test. Taken together, these experiments give a possible reason why there are various conflicting results in sign and goal tracking research. Contrary to the numerous different measures used to identify STs, the current work has demonstrated the necessity of an absolute criterion when classifying sign and goal tracking. Furthermore, STs are considered a drug addiction model. Misclassification of the behavior sign tracking may therefore slow the understanding of the mechanisms of drug addiction. Consequently, an accurate classification of STs is imperative for the future of drug research. It should also be noted that the current proposed t-test method can be adapted to other indices of sign and goal tracking and used in sign and goal tracking behavior that is not specific to rodents. The t score should more accurately identify STs, hypothesized to be most vulnerable to drug addiction. This novel tool may be of value for use in future drug addiction studies.

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CURRICULUM VITA

Beth Ann Rice

BIBLIOGRAPHICAL DATA

EDUCATION

M.S., University of Kentucky (expected, Aug. 2015)
Experimental Psychology: Behavioral Neuroscience & Psychopharmacology

B.S., Indiana University (Aug. 2012)
Psychology

POSITIONS

2012-Present	Research Assistant	Dr. Chana Akins	University of Kentucky
2013-Fall	Teaching Assistant	Dr. Steven Arthur	University of Kentucky PSY 215
2014-Spring	Teaching Assistant	Dr. Hui Chu	University of Kentucky PSY 215
2014-Fall	Teaching Assistant	Dr. Andrea Friedrich	University of Kentucky Honors PSY 215
		Dr. Steven Archer	PSY 215
2015-Spring	Teaching Assistant	Dr. Andrea Friedrich	University of Kentucky PSY 100
2015-Summer	Instructor		University of Kentucky PSY 311

FELLOWSHIPS, HONORS AND AWARDS

2011-2012	Student Research Fellowship, Indiana University Southeast
2012-2013	Obannon Scholarship, Indiana University Southeast
2012-2013	Arthur Alfonso Schomburg Graduate Fellowship, University of Buffalo

2013- 2014	Lyman T. Johnson Academic Year Fellowship, University of Kentucky
2014-2015	Lyman T. Johnson Academic Year Fellowship, University of Kentucky
2015-2016	Lyman T. Johnson Academic Year Fellowship, University of Kentucky

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PRESENTATIONS

Rice, B.A. and Akins, C.K. March 2013. Development of an avian model to study individual differences in drug addiction. Bluegrass Society for Neuroscience. Lexington, KY

Rice, B.A. and Akins, C.K. May 2013. Cocaine pre-exposure enhances sexual conditioning and increases extinction in male Japanese quail. Midwestern Psychology Association, Chicago, IL

Rice, B.A. and Akins, C.K. September 2013. Cocaine pre-exposure enhances sexual conditioning and increases extinction in male Japanese quail. Annual Pavlovian Society. Austin, TX

Rice, B.A. and Akins, C.K. March 2014. A novel statistical procedure for identification of individual differences of drug abuse. Bluegrass Society for Neuroscience. Lexington, KY

Nyerges, L. Rice, B.A. Husain, H. and Akins, C.K. April 2014.Development of an avian model to study individual differences in drug Addiction. NCUR, Lexington, KY

Rice, B.A. and Akins, C.K. May 2014. Development of an avian model for investigating phenotypes of drug addiction. Midwestern Psychology Association, Chicago, IL

Rice, B.A., Keller, P. and Akins, C.K. September 2014. Classifying sign and goal tracking using a T test in Pavlovian conditioning approach procedure. Annual Pavlovian Society, Seattle, WA

Rice, B.A. and Akins, C.K. May 2015. Quail prone to attribute incentive salience to a neutral-reward cue also attribute incentive salience to a cocaine cue. Midwestern Psychology Association, Chicago, IL

Edmiston, E.A., Rice, B.A., Akins, C.K. Effects of cocaine on sign and goal tracking behavior in male Japanese quail. Psychology Day, Lexington, KY

PUBLICATIONS

Akins, C.K., Gill, K., and Rice, B.A. (2015). Cocaine induces state-dependent learning of sexual conditioning in male Japanese quail. *Physiology & Behavior,* pp. 150-153.

Rice, B.A., Keller, P., Akins, C.K. (2015). Classifying Sign and Goal Trackers Using a T-test in a Pavlovian Conditioned Approach Procedure with Male Japanese Quail. (in preparation)

MEMBERSHIPS

American Psychology Association, Division 6, 2013-present
Bluegrass Society for Neuroscience, 2012-present
Midwestern Psychology Association, 2013-present
Pavlovian Society, 2013-present
Women in learning, 2013-present

SERVICE

Science Fair Judge, Veterans Park Elementary School, (2012)
Science Fair Judge, Veterans Park Elementary School, (2013)
Invited Speaker at Indiana University Southeast, (2013)
Abstract reviewer for National Conference on Undergraduate Research, (2013)
PSYCH Bowl Judge, Kentucky Psychological Association, (2014)
Invited Speaker at Indiana University Southeast, (2014)
Weird Science Night, Maxwell Elementary School, (2014)
Graduate Student Handbook committee member, University of Kentucky, (2014)
Undergraduate Poster judge, Kentucky Psychological Association, (2015)
Speaker at Bryan Station High School, (2015)