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EFFECTS OF CHEMOGENETIC INHIBITION OF DOPAMINE
TRANSPORTER- OR A2A-EXPRESSING NEURONS ON SPONTANEOUS
ACTIVITY AND MOTIVATION TO CONSUME A PALATABLE FOOD REWARD

BY

James D. Wherry

BA, Slippery Rock University, 2015

THESIS

Submitted in partial fulfillment of the requirements for
the degree of Master of Science in Psychology
in the Graduate School of Binghamton University
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Accepted in partial fulfillment of the requirements for
the degree of Master of Science in Psychology
in the Graduate School of Binghamton University
State of New York
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Abstract

Dopamine (DA) transmission in the striatum influences the motivated pursuit of rewarding stimuli. Pharmacological and opto- and chemo-genetic studies have suggested that the release of DA onto D2+/A2A+-expressing striatopallidal neurons, plays a role in this process. To determine the potentially dissociable roles of DA-releasing ventral midbrain and striatopallidal neurons on motivational processes, we employed double transgenic mice that expressed inhibitory DREADDs - designer receptors that are activated only by otherwise inert ligands - only in dopamine transporter (DAT) or A2A adenosine receptor (A2A) expressing neurons, allowing us to transiently inhibit either DA-releasing neurons (DATcre/DREADD) or striatopallidal neurons (A2Acre/DREADD) during various tests. In the first experiment, locomotor activity in a familiar environment was measured after mice received an injection of the DREADD ligand CNO (1 or 2.5 mg/kg) or vehicle. Both lines of mice exhibited decreases in spontaneous activity in response to the high dose of CNO. Second, voluntary consumption of sweetened condensed milk (SCM) was assessed. Both doses of CNO caused a significant decrease in SCM consumption in A2Acre/DREADD, but not DATcre/DREADD, mice. Finally, mice were trained and tested on a progressive ratio task, wherein increasing numbers of lever presses were required to obtain access to SCM. We found that CNO and Compound 21 (3 mg/kg) reduced active lever pressing in A2Acre/DREADD mice but not DATcre/DREADD mice. In none of these experiments were effects of CNO or Compound 21 detected in littermate control mice that were not double transgenic, supporting the idea that the effects observed were not due to back

metabolization of CNO or other “off target” effects. Thus, inhibition of A2A-expressing (presumably striatopallidal) neurons suppresses free and effortful reward pursuit and consumption; the degree to which this is attributable solely to impaired motor activity requires further study. Further studies will also assess the opposite effects of activating these same neural populations using excitatory DREADD (Gq) construct. Overall, these data do not support the idea that dopaminergic neural activity plays a major role in motivation for a palatable reward, but does implicate A2A-expressing neurons in both motor and motivational phenotypes.

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Effects of chemogenetic inhibition of dopamine transporter- or A2A-expressing neurons on spontaneous activity and motivation to consume a palatable food reward

Motivation to pursue and experience reward is an integral facet of everyday life and is thought to be driven partly by dopamine (DA) transmission in the brain.

Motivation is defined as the reasons for which an organism undertakes a specific behavior (Guay et al., 2010). Motivation can be further split into intrinsic (performing an action for its own sake) and extrinsic (engagement in an activity for some other gain).

There are several different ways in which motivation can be operationalized in humans, with tasks ranging in levels of physical and cognitive effort. However, the Progressive Ratio (PR) task allows for the measurement of motivation in nearly every species (Young & Markou, 2015). This paradigm requires a progressive increase in effort on the part of the subject in order to gain a reward and assesses the subject's "break point", when the work to be expended outweighs the value of the procured reward and reward pursuit, consequently, ends.

The idea that DA plays a role in motivation is derived from evidence implicating dopamine abnormalities in a number of disorders associated with marked changes in motivation. For instance, dopamine dysfunction is believed to play a role in reward seeking deficits often associated with schizophrenia, depression, obesity, and drug addiction. (Salamone, Correa, Farrar, & Mingote, 2007; Jong et al., 2015). Schizophrenia is marked by amotivation in a subset of patients (Young & Markou, 2015). Studies have found that patients who score poorly on scales of negative symptoms also show significantly lower scores in PR tasks in comparison to controls (Wolf et al., 2014). Similar results were found in patients with major depressive disorder, as measured by an effort-based decision-making paradigm (Park, Lee, Kim, Kim, & Koo, 2017).

While Schizophrenia and depression are marked by reductions in motivation, we find the opposite effect in drug addiction. Drug abuse is thought to hijack both motivational and executive function circuits through heightened levels of reward salience and habit formation (Koob & Volkow, 2016). Thus, researchers find high levels of PR responding for drugs of abuse in rodents, and this has become a successful method of measuring the reinforcing efficacy of clinical drugs (Richardson & Roberts, 1996). Similar findings have been found in rodent models of obesity, with obese mice showing higher levels of lever pressing for sucrose (Fleur et al., 2007). This is not surprising, as both food and drug related cues can activate similar neural structures and biochemical mechanisms (Cason et al., 2010).

Thus, deficits in motivation allow for insight into the mechanisms that drive motivation for reward. Several structures and pathways have been implicated in motivation, however, the specific neural mechanisms that modulate motivation remain incompletely defined. In the current experiment we wanted to gain further insight into DA transmission in the striatum, a structure that has long been connected with reward.

Circuitry of the Striatum

I. Anatomical divisions

In the study of motivation and reward, the striatum has become a structure of interest in several research ventures. The rodent striatum can be divided – anatomically – in a number of different ways. In one rubric, two major compartments, the ventral and dorsal striatum are recognized. While this division is thought to be anatomical rather than functional, with both structures receiving input from similar structures, it should be noted that the levels of innervation differ across the striatum. For example, the amygdala and

hippocampus heavily innervate the ventral striatum, but have less of a presence dorsally (Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). Differently, the dorsal striatum receives sensorimotor information from the frontal cortex and intralaminar thalamic nuclei (Voorn et al., 2004).

These striatal subregions can then be subdivided. The ventral striatum - or nucleus accumbens (NAc) - can be divided further into the core and shell (Soares-Cunha, Coimbra, Sousa, & Rodrigues, 2016) – a structure that, along with the bed nucleus of stria terminalis and the central amygdala – constitutes the extended amygdala network. The dorsal striatum can be split into the dorsolateral and dorsomedial striatum (Soares-Cunha et al., 2016).

There are functional differences associated with these fractionations of striatal anatomy. For example, consummatory behaviors and reward salience have often been demonstrated after functional manipulations of the ventral striatum (Kelley, 2004). Cognitive and executive functions are sensitive to manipulations of either the dorsal or ventral portions of the striatum (Packard & Knowlton, 2002). Several studies have found that both of these divisions have effects on behavioral flexibility and inhibition, however they seem to be associated with different aspects (Voorn et al., 2004). Neuronal populations in the ventral and dorsal striatum also show some electrophysiological differences. For example, the dopaminergic innervation suppresses glutamatergic input into the ventral striatum, however this is not found in the dorsal striatum (Nicola and Malenka, 1998). Thus, manipulations of DA transmission could have different implications across the striatum.

II. Glutamatergic innervation

Cortically originating projections to the NAc, which utilize glutamate as a neurotransmitter, influence several facets of reward and motivation. Specifically, motivation can be modulated through glutamatergic efferents from structures such as the medial prefrontal cortex (mPFC), the basolateral amygdala (BLA), and the ventral subiculum (vSub) in the hippocampus (Sesack & Grace, 2009). Communication between the mPFC and the NAc is thought to deal with the evaluation of subjective reward between differing stimuli and in selecting appropriate response strategies to gain a more salient reward (Sesack & Grace, 2009). The BLA also provides glutamatergic input to the NAc, causing longer lasting excitation. This direct projection is thought to deal with response to reward-predictive cues, drug reward, and palatable natural rewards (Haber, 2016). The last glutamatergic afferent comes from the Vsub. The Vsub, a structure whose output can be modulated by the amygdala and mPFC, is believed to code for affective and spatial stimuli through its projections to the NAc (French, Hailstone, & Totterdell, 2003). This is important for assessing the valence of reward in differing contextual situations (Sesack & Grace, 2009). It is important to note that these structures work in conjunction with one another in order to guide motivated behaviors. For example, the hippocampus is capable of gating excitatory projections from the mPFC, while the mPFC plays a role in modulating DA release in the NAc caused by BLA innervation (O'Donnell & Grace, 1995). Modulation by the NAc is also critical for changes in motivational behaviors.

III. Modulation of activity by the NAc

In the NAc, glutamatergic input from these cortical structures is directly influenced by ascending dopaminergic inputs from midbrain structures, including the

ventral tegmental area and *substantia nigra pars compacta*, allowing for the modulation of reward seeking responses. For example, input from the PFC is thought to be modulated primarily through dopamine D2 receptors in the NAc (Goto & Grace, 2005). Thus, when D2 receptors are activated by increased concentrations of synaptic dopamine, there is an attenuation of input from the mPFC which leads to a shift in response strategy (O'Donnell & Grace, 1994). Conversely, Vsub projections are upregulated through dopamine D1 activation in the NAc. This increase in communication between the structures seems to deal with reward selection, specifically when making the choice between salient and non-salient stimuli (Schultz, 1998).

IV. The mesolimbic pathway

While the input and modulation of glutamatergic afferents to the striatum are of importance, the mesolimbic pathway, connecting dopaminergic nuclei in the brain with the NAc, is a key mechanism in motivation and reward research. This pathway consists of DA projections from the VTA to the ventral striatum, which includes both the NAc and the olfactory tubercle (Ikemoto & Bonci, 2014). Levels of overall DA decrease during aversive events, or when an expected reward is not introduced (Schultz, 2007). The opposite effect is seen when subjects receive an unexpected, or better than expected, reward. This principal is known as the reward prediction error, and fluctuations in DA are thought to effect learning and reward seeking due to changes in reward processing (Wise, 2006). Decreases in DA are considered deficits in reward learning due to decreases in salience, rather than any dysfunction in general performance of the tasks (Dickinson, Smith, & Mirenowicz, 2000). Projections from the VTA to the prefrontal cortex and the amygdala also play a role in reward processing (Kelley & Berridge, 2002). Through

reciprocal connections, the NAc directly modulates innervation from the VTA, mainly through D1 activity (Yang et al., 2018). Furthermore, dopamine release into the NAc has the potential to modulate neurons of at least two distinct striatal projection systems, the direct and indirect pathway.

V. The direct and indirect pathway

The striatum is made up of several different cell types, including projection neurons and cholinergic and GABAergic local interneurons. GABAergic projection neurons, or medium spiny neurons (MSN), make up to 90-95% of the striatal neurons. These neurons project to either the globus pallidus internal and *substantia nigra pars reticulata*, or the globus pallidus external, creating the direct and indirect pathway, respectively (Stanley, Gokce, Treutlein, Sudhof, & Quake, 2016). The direct, or striatonigral, pathway is made up mainly of D1-expressing MSNs, whereas the indirect, or striatopallidal, pathway is made up of D2-expressing MSNs. Molecularly, D1 receptors activate Gs-coupled GPCRs which causes an increase in cytosolic cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA). PKA then recruits glutamate receptors to the surface, such as AMPA and NMDA, allowing for greater excitation of neurons in the direct pathway (Surmeier, Ding, Day, Wang, & Shen, 2007). D2 receptors interact with the Gi pathway, causing a decrease in cAMP and PKA through its reduction of adenylyl cyclase (De Mei, Ramos, Litaka, & Borrelli, 2009). This cascade causes the removal of AMPA receptors from the synapse, leading to a decrease in excitation of striatopallidal neurons (Hakansson, Galdi, Hendrick, Snyder, Greengard, & Fisone, 2006; Surmeier et al., 2007).

The direct pathway blocks inhibitory projections from the globus pallidus internal (GPi) and *substantia nigra pars reticulata* (SNr) to the thalamus. This causes disinhibition of the thalamus, leading to greater excitation of the motor cortex which allows for hyperlocomotion. Oppositely, the indirect pathway allows for greater excitation of the GPi and the SNr through disinhibition of the subthalamic nucleus. Thus, greater inhibition of the thalamus leads to less excitation of the motor cortex and less movement overall (Lee et al., 2016; Calabresi, Picconi, Tozzi, Ghiglieri, & Di Filippo, 2014). Previously, it was believed that there were analogous direct and indirect pathways in both the dorsal and ventral striatum. However, it has become evident that there is a lesser degree of dichotomy between D1 and D2 neurons in the NAc compared to the dorsal striatum (Soares-Cunha et al., 2016). Thus, we were interested in the manipulation of these pathways, mainly the striatopallidal pathway, in order to define their role in motivational behavior.

DREADD Technology

In order to gain temporal cell-specific control over these systems, DREADD (designer receptors exclusively activated by designer drugs) technology has been used. DREADDs allow for the activation of specific G-coupled receptor (GPCR) signaling pathways through an inert ligand, such as Clozapine-N-Oxide (CNO) or Compound 21 (C21), that have few or no other endogenous effects *in vivo*. Because endogenous GPCRs interact with several different ligands and are found in numerous cells, manipulating any of these pathways in a cell-type specific manner is not feasible (Zhu & Roth, 2014). Thus, a mutated human muscarinic receptor was created that no longer responds to endogenous agonist acetylcholine, but does respond to clozapine-N-oxide, a

metabolite of clozapine that would have *in vivo* effects at only these evolved receptors (Zhu & Roth, 2014). The DREADD system allows for the activation or silencing of neurons in specific populations in a time dependent manner, allowing for the identification of specific neuronal responses in behavior (Armbruster, Li, Pausch, Herlitze, & Roth, 2007).

In the current experiment, we used hm4Di DREADD receptors in order to silence target neuronal populations through Gi-signaling. This can be achieved through the inhibition of excitability and/or vesicular release of neurotransmitter from the presynaptic cell, in many cases through activation of the G-protein inwardly rectifying potassium channels (Roth, 2017). With this in mind, the DREADD technique allowed us to separate, and silence, pre- and postsynaptic DA neurons in the ventral midbrain and striatum, respectively. Specifically, we targeted and silenced DAT expressing neurons, a transporter localized presynaptically in DA releasing neurons. We also targeted A2A expressing neurons, a receptor known to be colocalized with D2 receptors in striatopallidal postsynaptic neurons (Beggiato et al., 2014).

DREADD mice were generated through the use of the transgenic breeding approach, as opposed to the viral vector approach. This paradigm was chosen due to several benefits, such as: the ability to test more mice over a shorter period of time, it being a far less invasive approach, and the transgenic mouse allowing for a greater overall anatomical coverage during treatment. (Smith, Bucci, Luikart, & Mahler, 2016). Furthermore, transgenic lines allow for identical expression of DREADDs across all cells of interest, allowing for less variability between subjects. However, by choosing a transgenic approach, we lose the ability to anatomically localize DREADD expression, as

the viral technique allows for strong expression in specific structures and subpopulations of cells (Burnett & Krashes, 2016). Viral methods also allow for flexibility in when the DREADD is introduced into the genome. This can avoid transgenes having an effect on developmental processes. Nevertheless, there can be a number of issues with overall spread and virus transduction rate (Burnett & Krashes, 2016). This can be due to differences in diffusion rates across viral studies, and nonuniform transfection of neighboring cells in response to viral injection. This can result in the actual number of infected neurons varying greatly across animals that are used in a singular study (Burnett & Krashes, 2016). Something else that needs to be taken into account is the amount of time the study lasts. Because our study lasted several months, a transgenic approach was a better approach, as viral expression can last a shorter amount of time (Smith, Bucci, Luikart, & Mahler, 2016).

A final variable in studies involving DREADDs is the dosage of CNO administered. Doses of 1mg/kg and 2.5mg/kg of CNO were chosen for the current studies based on previous research done in the field (Robinson et al., 2014; Warthen et al., 2016). Doses vary across studies based on expression levels, species of animal, the length of experimentation, and overall cell type that are expressing the DREADD (Smith, Bucci, Luikart, & Mahler, 2016). However, several studies have found success with the use of 1mg/kg CNO (Ferguson et al., 2011; Chang, Todd, Bucci, & Smith, 2015). Other studies have used up to 20mg/kg of CNO without observing any side effects in control subjects (Mahler et al, 2014). Because there are differences in CNO dosage across studies, we performed dose response curves before choosing doses that were implemented in the current study. With this in mind, researchers used DREADD technology in order to

transiently silence midbrain DA projections or postsynaptic D2/A2A expressing striatopallidal neurons; allowing for researchers to dissociate the importance of both these factors in motivational behavior, as measured through several behavioral assays.

Locomotor Activity

Of course, when manipulating striatal DA neurons, it is important to analyze the effects this has on spontaneous activity and movement. Traditionally, studies have found that activation of D1 or D2 receptors in the NAc has led to an increase in locomotor activity, while antagonism of either receptor led to the opposite effect (Baldo, Sadeghian, Maria, & Kelley, 2002; Dreher & Jackson, 2006) This makes anatomical sense, being that greater activation of D1 direct pathway neurons leads to D1 mediated activation of the striatonigral pathway, and activation of D2 leads to Gi mediated silencing of the striatopallidal pathway (Surmeier et al., 2007)

It follows that these pathways were split into the classic “go/no-go” dichotomy, and several studies have repeated these findings, whether it be through optogenetics, reversible neurotransmission blocking, or DREADDs (Freeze, Kravitz, Hammack, Berke, & Kreitzer, 2013; Hikida, Kimura, Wada, Funabiki, & Shigetada, 2010; Lemos et al., 2016) However, other studies have found that the involvement of these two systems in movement is more complicated. For example, Cui and colleagues (2014) showed that both the direct and the indirect pathways exhibit neuronal activation when motor movement takes place. This shows support for previous evidence which purports that movement requires coordinated activation of both direct and indirect pathways, sometimes simultaneously (Chan, James, & Yung, 2005).

Taking this into account, we measured total distance traveled in locomotor chambers after the transient inhibition of pre- and postsynaptic DA neurons. We hypothesized that presynaptic DA inhibition in DAT^{cre}/DREADD double transgenic mice would lead to lower levels of overall ambulation – through lowering DA release and activation of postsynaptic D1 and D2 receptors, while silencing of striatopallidal neurons in A2A^{cre}/DREADD mice would lead to increased locomotor activity (by inhibiting striatopallidal neurons). After locomotion testing, researchers measured consumption of a natural reward.

Voluntary Consumption of a Natural Reward

Striatal DA has an influence on motivation to seek and consume natural rewards. This finding has been repeated for a large spectrum of stimuli ranging from food to social interactions (Cannon & Bseikri, 2004). The mesolimbic system plays a role in this process, involving innervation of the striatum by the VTA, releasing the neurotransmitter DA (Ikemoto & Panksepp, 1999). Event-related increases in DA release are associated with elicited approach behaviors, which can entail anything from exploratory tendencies to performing trained behaviors in order to gain the reward (Ikemoto, Yang, & Tan, 2015). On the other hand, reductions in DA in the striatum can suppress approach behavior and conditioned place preference (Ikemoto & Panksepp, 1999; S. Shippenberg, 1993). Fluctuations in DA are associated with reward delivery or omission when expectations are violated (Bassareo & Chiara, 1999). This fits well with the theory of reward prediction error; a mechanism which plays a large role in motivation and learning (Glimcher, 2011). As learning progresses and expectations are no longer violated, DA release decreases as sessions are repeated for natural rewards. However, similar

decreases in DA were not found in studies using psychostimulant drugs as a reward (Ikemoto & Panksepp, 1999; Ikemoto et al., 2015).

Dopaminergic modulation of approach behavior is thought to be driven, in part, by D1 and D2 expressing striatonigral and striatopallidal neurons. Microinjections of D1 antagonists into the ventral striatum are sufficient to induce conditioned place aversion, and the inhibition of VTA-ventral striatum afferents have a similar effect (Liu, Shin, & Ikemoto 2008; T. S. Shippenberg, Huber, & Herz, 1991). Conversely, transient optogenetic activation of either the ventral striatum or VTA DA neurons results in conditioned place preference (Ilango et al., 2014). Antagonism of D1 or D2 receptors also lead to devaluation of a sucrose reward, providing evidence that activation of both receptors is required for reward (Clark & White, 1987). This is due partly to the decrease in nigrostriatal neuronal firing and an increase in striatopallidal neuronal firing, respectively. This pattern has been found to produce reductions in both drug taking and drug seeking behaviors, which could of course have therapeutic significance (Bock et al., 2013).

DA neurons have also been implicated in specific consummatory behaviors. Chemogenetic inhibition of VTA DA neurons caused a decrease in time spent consuming palatable food, and an overall increase in sleep and sleep preparation behaviors, including nest building (Eban-Rothschild, Rothschild, Giardino, Jones, & de Lecea, 2016). Dopaminergic modulation of D2 receptors, in particular, may relate to food consumption, as D2 receptor binding/availability in the striatum is decreased in human obesity and in relevant rodent models (Friend et al., 2016). While this may be the case, several studies transiently inhibiting DA pathways have found that, while there are decreases in overall

approach behaviors, there is no change in amount of reward consumed (Eban-Rothschild et al., 2016; Ikemoto & Panksepp, 1996; Liu, Shin, & Ikemoto, 2008), suggesting that DA plays more of a role in anticipatory aspects of consumption, rather than in consummatory behaviors themselves.

In a test of voluntary consumption of natural reward, we predicted that inhibition of presynaptic DA neurons would lead to a decrease in overall consumption, driven by delay in approach behaviors in comparison to control animals. Oppositely, inhibition of postsynaptic D2 neurons would lead to increased consumption due to an increased number of approach behaviors. After the measurement of voluntary consumption, we were interested in how these manipulations effected motivation in a task that involved effort expenditure to obtain access to food.

Measures of Motivation in Operant Responding

While exploratory behavior and reward salience plays a role in voluntary consumption of a natural reward, higher levels of motivation are needed in order to effortfully respond to gain a reward. This idea has been described as work-related response costs and describes the amount that one is willing to work in order to gain a reward (Salamone et al., 2016). This tends to be on a sliding scale, with more salient rewards meriting higher levels of effort. Unsurprisingly, DA is thought to play a key role in this process, as well.

Levels of DA in the striatum have been found to become more important as tasks begin to require more effort (Aberman & Salamone, 1999). In fact, one study found that depletions of DA in the NAc have little effect on operant responding for a palatable food when minimal work is required. However, when an increasing number of lever presses

was required for reward, reduction of DA caused significantly lower levels of responding (Salamone, Correa, Farrar, & Mingote, 2007). This provides evidence that striatal DA plays some role in decision making when analyzing work related costs. There are several hypotheses as to why this may be the case, with many arguing whether DA in the striatum leads directly to more effortful behavior, or whether it merely increases the probability of making a response (Floresco, St. Onge, Ghods-Sharifi, Winstanley, 2008; Nicola, 2010). Another hypothesis merits that the behavioral tests itself is what matters. Nicola (2010) found that DA in the NAc was only important when reinforcement contingencies adjust during a task, as in a progressive ratio test wherein response requirements increase over time. However, DA levels become negligible if the task to gain the reward remains static (Nicola, 2010).

Manipulations of direct and indirect pathways also have a large effect on motivation for effortful behaviors. For example, either antagonism of D1 or D2 receptors led to reduced lever pressing for a palatable reward and increases in consumption of a freely-available but less palatable reward (Nowend, Arizzi, Carlson, & Salamone, 2001; Koch, Schmid, & Schnitzler, 2000). Furthermore, D2 receptor knockdown in the VTA has led to increases in responding for sucrose solution, thought to be driven mainly through reduction of autoreceptors which normally inhibit neuronal firing and dopamine output (Jong et al., 2015). However, overexpression of postsynaptic D2 receptors in the striatum had opposite effects, with mice showing deficits in lever pressing for evaporated milk. This deficit was reversed through rescued Gi signaling, which effectively inhibited striatopallidal neuronal output using an inhibitory DREADD. Interestingly, the same effect was found in control mice expressing natural levels of D2 receptors as well,

showing that increased Gi signaling in normal functioning mice can also increase motivation (Carvalho et al., 2016).

Thus, in the current study, we tested mice on a progressive ratio operant task, in which the number of lever presses needed for reward increased after every trial. We hypothesized that inhibition of presynaptic DA neurons (DAT^{cre}/DREADD) would lead to a reduced number of lever presses, while inhibition of postsynaptic D2 neurons (A2A^{cre}/DREADD) in the striatum would cause a significant increase in operant responding.

Methods

Breeding

B6.SJL-Slc6a3^{tm1.1(cre)Bmn}/J mice hemizygous for IRES-cre(DAT^{IRES^{cre}}) knock-in allele were initially bred with B6N.129-Gt(ROSA)26Sor^{tm1(CAG-CHRM4*,-mCitrine)ute}/J mice; containing the Rosa-CAG-LSL-HA-hM4Di-pta-mCitrine (R26-hM4Di/mCitrine) conditional allele. A subset of these offspring contained both the IRES-cre and R26-hM4Di/mCitrine allele, showing effective cre-dependent removal of the stop cassette, and allowed for the transient silencing of pre-synaptic DA neurons containing the Gi-DREADD receptor through CNO or C21 treatment. Single transgenic mice, mice only containing the IRES-cre or R26-hM4Di/mCitrine allele, were tested in conjunction with wildtype mice, as littermate controls.

B6.FVB(Cg)-Tg(Adora2a-cre)KG139Gsat/Mmucd mice heterozygous for Tg(Adora2a-cre)KG139Gsat allele were also bred with B6N.129-Gt(ROSA)^{26Sortm1(CAG-CHRM4*,-mCitrine)ute}/J mice. Offspring containing both alleles showed transient silencing of

D2/A2A containing neurons, when treated with CNO or C21. Again, single transgenic and wildtype mice were used as controls.

Husbandry

The majority of mice used in the study were socially housed throughout the duration of experiments, however, some were singly housed due to conspecific aggression. All mice were housed on a 12-hour light/dark cycle (lights on at ~7AM). All water and food were provided *ad libitum* in the home cage until the initiation of operant conditioning, at which time the subjects had restricted access to food. All animals used in experimentation were bred in house and colony rooms were kept at a constant temperature (~68 F).

Drugs

DREADD receptor agonist 8-Chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzyl[b,e](1,4)diazepine N-oxide (Clozapine N-oxide) was purchased from Sigma Aldrich and dissolved in 0.9% sodium chloride solution and .5 μ L or 1.25 μ L dimethyl sulfoxide, dependent on dose of CNO. 11-(1-Piperazinyl)-5H-dibenzo[b,e][1,4]diazepine dihydrochloride (Compound 21) was purchased from Hellobio and dissolved in 0.9% sodium chloride solution.

Locomotor Activity:

Locomotor activity was measured in mice during the light phase using the Seamless Open Field Arena for Rat (17" L x 17" W x 12" H, ENV-515S) produced by Med Associates Inc. (St. Albans, VT). The apparatus contained clear Plexiglas walls and included a ventilated cover to prevent escape. Total distance traveled was measured by three sets of photobeam detectors (two providing an X-Y coordinate plane on the cage

floor and a third for measuring rearing or jumping). On the first day of testing, mice were weighed, placed into the apparatus, and allowed to acclimate over a 60-min period. On the second day, mice were given a saline injection (10ml/kg of a 0.9% sodium chloride solution, IP) and again placed into the apparatus for 60-min. On the subsequent testing days, mice were given an IP injection of either a 0.9% sodium chloride solution, 1mg/kg CNO, or 2.5mg/kg CNO. At the time of testing, mice were weighed and placed into the open field for a 15-min acclimation period. Then, the program was paused; mice were retrieved from the arena and given an IP injection. They were then placed back in the apparatus for an additional 90-min. The order of drug treatments was counterbalanced across subjects using a cyclic Latin square design.

Voluntary Consumption:

Voluntary consumption of a natural reward, 10% sweetened condensed milk (SCM; v/v in tap water), was measured via lickometers in the Scurry Activity Monitor (9.3", 13.9".7.7", 80820S), produced by Lafayette Instrument (Lafayette, IN). Each Scurry box contained two 50 mL bottles, one containing water and the other containing SCM solution. Across at least eight days of testing, mice were placed in the apparatus and given free access to both bottles for 120-min. Position of the water and SCM bottles were varied across all testing days to control for position preference. Licks/s were measured for each bottle by a computer. All licking data was analyzed to ensure animals had reached a stable baseline of consumption before subsequent drug testing. After stable consumption was reached, mice received IP injections of saline, 1mg/kg CNO, 2.5 mg/kg CNO, or 3 mg/kg C21 on different test days, assigned to each mouse in a counterbalanced manner according to a Latin square. Each dose was administered twice,

and no dose was given in succession across two testing sessions. The number of doses administered for each mouse was decided *a priori* and was instituted because of occasional, unexpected malfunctions in the Scurry system. We reasoned that by conducting two sessions at each dose, we would get at least one valid data point per dose. Outlier data determined whether such an error occurred, and these data points were removed from analyses. When no malfunction occurred at either dose, the data from the two sessions were averaged together.

Operant Testing:

After the first two assessments were completed, animals were introduced to a schedule of limited access to chow in their home cages. All mice began the experiment at ~7:30AM (within 1-h of lights on) and were fed their daily allocation of normal chow at ~12:30PM (about 1-h after testing of all subjects completed). The amount of food each animal was fed daily was titrated in order to achieve body weights that were ~85% of their free feeding weights, in order to motivate operant responding. Operant chambers were acquired through Med Associates; they were 8.5" L x 7" W x 5" H (21.6 x 17.8 x 12.7 cm). All tests were run with background white noise provided and with the house light on.

I. Habituation

On the first day, mice were habituated to the operant chambers for 60-min. No programmed events occurred.

II. Magazine Training

In the first stage of magazine training, mice were presented with a reinforcer of 20-21 μ L of SCM on a variable time schedule, with an inter-trial interval (ITI) ranging from 1-108s. The ITI timer was initiated after the subject retrieved the liquid from the previous trial. These sessions ended after 30-min or after 20 reinforcers had been obtained, whichever came first. Day two of magazine training was nearly identical to the first, however initiation of the ITI was not dependent on retrieval of the previous reinforcer.

III. Pavlovian Autoshaping

After magazine training, subjects completed one session of autoshaping, wherein reinforcer delivery was paired with insertion of the left lever. The left lever was inserted for 5s before being retracted, followed by the delivery of 20-21 μ L of SCM. Importantly, mice did not need to interact with or actuate the lever in order for the reinforcer to be delivered; it merely served as a reward predictive cue. The ITI was set to an average of 60 s per trial, and sessions lasted for 60-min.

IV. Continuous reinforcement training

In stage four, mice were reinforced with 20-21 μ L of SCM for each lever press completed on the left lever (the right lever was never inserted). After every two reinforcements earned, the lever was retracted for an average ITI of 30-s. These testing sessions continued daily until the subject gained 60 rewards in two consecutive testing days. Each session ended after 60 min or after 60 reinforcers were earned, whichever came first.

V. Random interval training

In this stage, the left lever was presented; all lever presses were counted, but only the first press that occurred after a random interval (RI) had elapsed, was reinforced. After every second trial completion the lever was retracted for an ITI averaging 20-s. In the first session, mice were required to obtain 40 reinforcers with an RI averaging 3-s. If 40 reinforcers were earned in the span of 60 min, mice were stepped on to a session with a longer RI. Mice eventually advanced through training stages involving an RI averaging 10, 15, and 20 seconds. When they successfully completed the last stage, they were advanced to Progressive Ratio testing.

VI. Progressive Ratio Task

In the last stage, mice completed a progressive ratio task, in which the number of lever presses required to trigger reinforcement were doubled after every successive reinforcer delivery. Sessions were terminated after 120 min, or after a mouse failed to respond for 3-min. After every second schedule completion, the lever was retracted for an average of 20s. Sessions were repeated until the subject reached stable baseline responding, calculated by each mouse having less than an 80% response variation across two sessions. Once subjects reached stable responding, drug treatment began. Using a Latin square design, mice were put on a schedule of drug treatment, receiving either saline, 1mg/kg CNO, or 2.5mg/kg CNO. Mice received IP injections directly before testing began, and each dose was tested twice in each subject. When this study began, it was unclear how variable we could expect the data to be. In order to address this point, each dose was tested twice; however, identical doses were not repeated in succession. Mice received treatments only after 48 hours had elapsed since the previous injection. Mice received treatments only after 48 hours had elapsed since the previous injection.

After mice were tested using CNO, researchers began treatment with C21. Similarly, mice received treatment with saline or C21 twice across two differing test sessions.

Statistical Analysis

Data for each variable was analyzed by an ANOVA using GraphPad Prism 6 (San Diego, CA). Independent variables were genotype and treatment, and dependent variables were total distance traveled, number of licks, and number of lever presses in the locomotor, free intake and operant conditioning tests, respectively. In order to first examine the data for baseline differences in behavior between genotypes in our different behavioral assays, we conducted an ANOVA with genotype as the between subjects factor; Tukey's post-hoc was enlisted to decompose any main effects detected. We next conducted an omnibus ANOVA with genotype as a between subjects factor and treatment as a within subjects factor, again with Tukey's post hoc tests to decompose main effects or interactions. Because of the hypothesis-driven nature of our study, we used one-way ANOVA in order to analyze differences across treatment in A2A/DREADD animals and DAT/DREADD animals, separately, even in the absence of a genotype x treatment interaction. A Dunnett's multiple comparison test was then used to detect treatment effects in these two lines. In all cases, a Geisser-Greenhouse correction was used when the assumptions of sphericity were violated.

In the specific case of the measures of consummatory behavior across time, an omnibus ANOVA including genotype as a between subjects factor and treatment and time as within subjects factors was used. After finding a significant interaction, we then used Tukey's multiple comparisons test.

Results

Experiment 1- Locomotor Activity

The sample of animals used in these analyses are located in figure 8.

I. Baseline differences

First, we used an ANOVA in order to detect differences in total distance traveled between genotypes at baseline. For this test, genotype was used as a between subjects factor, and resulted in a main effect of genotype ($F(5, 122)=6.149$, $p<0.01$). A Tukey's multiple comparison test was then used. When injected with saline, A2A/DREADD mice ambulated less than wildtype ($t(122)= 5.503$, $p<.05$) and DATcre ($t(122)= 6.778$, $p<.05$) mice. Similarly, DAT/DREADD mice ambulated less than DATcre controls ($t(122)= 5.348$, $p<.05$) when treated with saline (figure 1). Thus, there appeared to be baseline differences in spontaneous ambulatory activity in both double-transgenic lines when saline was delivered.

II. Exploratory analyses

Next, an omnibus ANOVA was used in order to analyze the effects of treatment on ambulation across different genotypes. In this test, genotype was used as a between subjects factor and treatment as a within subjects factor. The omnibus ANOVA detected main effects of both genotype ($F(5,122)=10.18$, $p<.01$) and treatment ($F(2,244)=11.57$, $p<.01$). However, there was no interaction found between the two factors.

III. A priori hypotheses

Lastly, we wanted to analyze whether treatment with CNO differed from treatment with saline in our double transgenic animals. We hypothesized that CNO treatment in DAT/DREADD animals would decrease locomotion in comparison to saline

treatment. Oppositely, we predicted that CNO treatment would increase locomotion in A2A/DREADD animals in comparison to saline. An ANOVA was implemented to analyze these hypotheses, using treatment as a within subject factor. DAT/DREADD mice showed a main effect of treatment ($F(1.368, 31.46) = 18.43, p < .01$). Dunnett's multiple comparisons test showed that Mice ambulated significantly less when treated with 1mg/kg CNO ($t(23) = 3.851, p < .05$) and 2.5mg/kg CNO ($t(23) = 5.359, p < .01$) in contrast to saline. A main effect of treatment was also found in A2A/DREADD mice ($F(1.764, 35.28) = 3.289, p < .01$). This effect came from differences in ambulation between 1mg/kg CNO and 2.5 mg/kg CNO ($t(20) = 2.986, p < .05$).

Experiment 2- Voluntary Consumption of SCM

The sample used in these analyses are located in figure 9.

I. Baseline differences

First, we wanted to see if there were any differences in baseline number of licks between all genotypes. This was analyzed through one-way ANOVA with genotype used as a between subjects factor. The ANOVA found no main effect of genotype ($F(5,70) = .9493, p > .05$). (figure 3). This provides evidence that groups of mice did not differ at baseline in their levels of licking behavior.

II. Exploratory analysis

Next, an omnibus ANOVA was run in order to analyze the effects of saline and CNO treatment on licking behavior across genotype. In this analysis, genotype was used as a between subjects factor, and treatment was used as a within subjects factor. This

analysis yielded a main effect of genotype ($F(5,176)= 2.774, p<.05$). However, no main effect of treatment nor any treatment x interaction was found.

We were also interested in differences in the expression of licking behavior across time in each treatment session. To find whether genotypes differed in this behavior, an omnibus ANOVA was run containing genotype as a between subjects factor and treatment and time as within subjects factors. We found an interaction of genotype and time point ($F(155,1784)=1.613, p<.01$), along with main effects of both genotype ($F(5,1784)=8.352, p<.01$) and time bin ($F(5,1784)=20.10, p<.01$). A Tukey post-hoc showed that, after treatment with 1 mg/kg CNO, A2A/DREADD had a significantly lower level of consumption in the first 15 minutes of testing in comparison to A2Acre ($t(1784)=6.653, p<.05$), wildtype ($t(1784)= 5.787, p<.05$), and DAT/DREADD ($t(1784)=7.541, p<.05$) (figure 5). A similar effect was found at the 2.5mg/kg dose of CNO, with A2A/DREADDs consuming less than A2Acre ($t(1784)=5.949, P<.05$) and DAT/DREADD ($t(1784)=6.825, p<.05$) in the first 15 minutes of testing (figure 5).

III. A priori hypotheses

Lastly, we wanted to test whether the effects of treatment on licking behaviors in our double transgenic animals. We predicted that CNO would increase licking in A2A/DREADD mice, and decrease licking in DAT/DREADD mice compared to saline. Hypotheses were analyzed through an ANOVA which used treatment as a within subjects factor. We found a main effect of treatment in A2A/DREADD mice ($F(2, 53)=11.7, p<.05$), showing that saline resulted in a significantly higher number of licks in comparison to 2.5mg/kg CNO ($t(53)=4.582, p<.05$), but not 1mg/kg CNO. This shows

that the high dose of CNO results in suppression of consummatory behavior, but this is not true of the lower dose. Furthermore, no differences in licks were found in DAT/DREADD mice across all treatments.

Experiment 3- PR Data

The sample of animals used in these analyses are located in figure 10.

I. Baseline differences

First, we analyzed whether there were any differences in baseline levels of responding between the genotypes. This was analyzed by one-way ANOVA with genotype as a between subject factor. The ANOVA showed a significant main effect of genotype in baseline responding ($F(5, 32)= 2.923, p<.05$). A Tukey's multiple comparison test found that DAT/DREADD animals registered a lower number of lever presses in comparison to A2Acre animals ($T(32)= 5.062, p<.05$). However, no differences were found between wildtype and transgenic animals.

II. Exploratory analyses

Next, an omnibus ANOVA was run in order to analyze changes in lever pressing across treatments. This was done by using genotype as a between factor, and treatment as a within factor. Percent change from baseline lever pressing was used as the dependent variable across each treatment. The ANOVA showed main effects of both genotype ($F(5,32)=9.051, p<.01$) and treatment ($F(3,96)=2.974, p<.05$), but no interaction was detected

III. A priori hypotheses

Lastly, we wanted to see if there were any differences in responding in A2A/DREADD and DAT/DREADD mice after CNO and C21 treatment when compared to saline treatment. We hypothesized that A2A/DREADD mice would register higher amounts of lever presses after treatment with both doses of CNO and C21 compared to saline. We also predicted that DAT/DREADD mice would lever press fewer times after CNO and C21 treatment than saline treatment. An ANOVA was run using treatment as a between subject factor, which found that A2A/DREADD mice showed a main effect of treatment ($F(1.554, 12.43) = 13.0, p < .01$). A Dunnett's post-hoc revealed A2A/DREADD mice showed reduced operant responding when treated with 1mg/kg CNO ($t(8) = 3.344, p < .05$), 2.5mg/kg CNO ($t(8) = 4.582, p < .05$) or C21 ($t(8) = 5.083, p < .05$), as compared with saline. A main effect of treatment was also found in DAT/DREADD mice ($F(2.315, 25.46) = 3.306, p < .05$). A Dunnett's multiple comparison test revealed a significant decrease in responding when treated with C21 in comparison to saline ($t(11) = 2.916, p < .05$)

Discussion

Inhibition of pre-synaptic DA neurons in the striatum has been shown to lead to changes in locomotion, voluntary consumption of palatable foods and operant responding indicative of motivation (Baldo et al., 2002; Eban-Rothschild et al., 2016; Nowend et al., 2001). Antagonism of D2 receptors has a similar effect, showing that this receptor in the striatum is integral for tasks involving motivated and consummatory behaviors (Clark & White, 1987). Our project looked to dissociate the actions of pre-synaptic DA expressing neurons and postsynaptic D2/A2A expressing striatopallidal neurons, using a DREADD technique that allowed for the transient silencing of pre- or postsynaptic DA neurons

during specific behavioral paradigms. This allowed us to analyze the roles of each neuronal population in these behaviors.

Locomotor Behavior

After testing mice in an open field arena after treatment, we found that there were baseline differences between controls and double transgenic mice, showing that DAT/DREADD and A2A/DREADD mice exhibit less spontaneous activity than their single transgenic and wildtype counterparts. These effects could be due to the additive genetic effect of the two transgenes (Matthaei, 2007). While this may be the case, CNO treatment was associated with significant decreases in DAT/DREADD mice, and inactivity to a lesser degree in A2A/DREADD mice compared to saline, though this result was not significant. The lack of potentiation in locomotion after postsynaptic Gi-DREADD activation confined to A2A-expressing cells was surprising, as previous studies have found similar manipulations to increase activity (Friend et al., 2016; Zhu, Ottenheimer, & DiLeone, 2016). There were no differences in locomotion in A2A/DREADD mice after treatment with saline compared to CNO. However, locomotion increased when double transgenic mice were treated with a lower dose of CNO versus a higher dose.

Different effects were observed for DAT/DREADD mice; in this line, either dose of CNO significantly reduced total distance traveled in contrast to saline. The effects of CNO on DAT/DREADD mice fit nicely with previous experiments which show that disruption of communication between midbrain and striatal DA neurons results in reduced ambulation (Durieux, Schiffmann, & Dexeerd, 2011; Bateup et al., 2010; Kravitz et al., 2010). This is congruent with the nigrostriatal pathway's and striatum's

roles in movement, which merits that DA is needed in order to disinhibit the thalamus, and inhibit the indirect pathway, allowing for greater overall ambulation (Wall, De La Parra, Callaway, & Kreitzer, 2013). This matched with our hypothesis, which predicted that overall locomotor activity in DAT/DREADD mice would decrease after treatment with CNO. However, we did not see an expected increase in ambulation in A2A/DREADD mice. Because both the DREADD and D2 receptor are Gi- coupled, we expected that decreasing the output of striatopallidal neurons would allow for less inhibition of the thalamus, and thus the motor cortex. However, due to the high degree of striatal excitation by activation in A2A cells, compensatory action could be at fault. For example, GABAergic inhibition of the direct pathway by interneurons in the PFC could be increased due to overexcitation (Rock, Zurita, Wilson, & Apicella, 2016). This could, in turn, block the effects of inhibiting the striatopallidal pathway.

Voluntary Consumption

We tested mice in a voluntary consumption paradigm to gauge the effects of CNO on consuming a salient reward when little effort was required. We found that DAT/DREADD mice were not affected by CNO treatment and did not differ in levels of registered licks between the DREADD agonist and saline. This was surprising, as we assumed that lower levels of dopamine would result in lower amounts of movement and approach behaviors, resulting in less consumption of SCM. Indeed, previous studies have found that the VTA is integral in consummatory behaviors (Meye & Adan, 2014). However, as discussed previously, manipulations of DA levels do not affect reward behaviors when effort is not required (Salamone, Correa, Farrar, & Mingote, 2007). Thus, it is possible that deficient levels of DA would not have any effect on overall

intake. This being due to the task not requiring enough effort in order for the decrease of DA to have any noticeable effect. Furthermore, other receptors located on pre-synaptic DA neurons are also being affected by the transient silencing of the Gi-DREADD. For example, Leptin receptors on VTA DA neurons play a major role in reducing overall food consumption, and inhibition of these neurons can lead to increases in food intake (Hommel et al., 2006). Though this is not the focus of the current experiment, it shows that other mediators of food consumption are disrupted when midbrain DA neurons are silenced.

We also found that A2A/DREADD mice registered less licks when treated with saline in comparison to 2.5 mg/kg CNO. This result did not coincide with our hypothesis, as we predicted that increases in DA would allow for greater approach behaviors, and thus greater overall consumption. Interestingly, previous studies have found that Gi-DREADD mediated silencing of D2 neurons does not have any effect on food consumption (Zhu, Ottenheimer, & DiLeone, 2016). However, it is a possibility that increased DA due to striatopallidal neuronal inhibition did not have the hypothesized effect due to the principal of reward prediction error described previously. Past research has found that overall DA levels decrease as rewards are given consistently, and DA transmission becomes less important as the environment and actions to gain reward are not flexible (Ikemoto & Panksepp, 1999; Ikemoto et al., 2015; Nicola, 2010). This explanation fits, as mice were tested on voluntary consumption for a total of 14 days with few days off in between. Thus, although both hypotheses were unsupported, it is possible this is due to the task requiring little effort and masking the effect of DA levels on motivational behaviors.

Operant Conditioning

Lastly, we wanted to measure how DA manipulations affected motivational behavior on a progressive ratio task. Previous studies have found that striatopallidal neuronal inhibition led to increases in lever pressing and operant responding (Gallo et al., 2018; Carvalho et al., 2016). However, CNO treatment seemed to have opposite effects in the current experiment, as lever pressing decreased significantly in A2A/DREADD mice after CNO treatment. A2A/DREADD mice also responded less after treatment, unlike control lines that were insensitive to CNO or C21.

Another explanation for why this may be the case is compensatory inhibition from other structures, mainly GABAergic neurons which make up a newly discovered corticostriatal pathway (Rock et al., 2016). Recent studies have found that long range GABAergic projecting neurons from the motor cortex are able to directly modulate output of both the direct and indirect pathway through pvalbumin and somatostatin positive interneurons (Melzer et al., 2007; Rock et al., 2016). While no research has identified the effects of this inhibitory pathway on motivation, it is possible that hyperactivity caused by the inhibition of the striatopallidal pathway could cause greater input from GABAergic interneurons. Furthermore, these effects were not found in DAT/DREADD mice, as responding remained stable across CNO and saline treatment.

Activity vs. Motivation

One possible explanation for the noted decreases in motivation to consume a freely available or effortfully obtained reward in A2A/DREADD mice could be an overall suppression of motor activity, though we think this is not a reasonable interpretation of the effects of the lower dose of CNO in A2A/DREADD animals.

Firstly, ambulatory and consummatory activity was only affected in A2A/DREADD mice after the high, but not low, dose of CNO (Figure 1 and 4), while both doses affected operant responding (Figure 7). Second, the low dose of CNO specifically attenuated the binge-like burst of SCM intake in the first 15-30 min the voluntary consumption test, while having no effect on the relatively high levels of intake that persisted afterwards (Figure 5). Third, CNO powerfully suppressed motor activity in DAT/DREADD animals (Figure 1), but had no effect on motivation in these same animals (Figures 5 and 7). Thus, these data support the idea that there is a mechanistic dissociation between motor activity and motivated reward pursuit and consumption, with inhibition of dopaminergic neurons primarily affecting the former, and inhibition of striatopallidal A2A+ neurons primarily affecting the latter.

Limitations

One limitation of our approach involves the use of CNO as a ligand to activate DREADD receptors. The inert effects of CNO have also been called into question, as a study has shown that CNO is not capable of crossing the blood brain barrier, but instead converts back to clozapine through reverse metabolism (Gomez, et al., 2017). While this may be the case, this had little effect on motivated behavior in control animals, only affecting double transgenic animals that express the DREADD receptors. The notable exception was the observed effect of CNO on motor activity in wildtype mice (Figure 1); since these effects were modest and since they were not observed in any other control lines (DREADD or cre only lines), it is likely true that this is not a reproducible effect. Furthermore, we made use of C21, another DREADD agonist, and found synonymous

results with CNO in our progressive ratio task, suggesting that the off target effects of CNO or its metabolites are not a significant concern in our studies.

Although the transgenic technique has several benefits in comparison to a viral technique, it does have some disadvantages, mainly due to the anatomical range of effects. In our lines, any neurons expressing DAT or A2A receptors would be silenced when treated with CNO, including neurons outside of the nigrostriatal and/or mesocorticolimbic pathways. Though it is well known that DAT and A2A receptors are concentrated pre- and postsynaptically, respectively, and are heavily concentrated in the ventral midbrain and striatum, respectively, they are expressed elsewhere in the nervous system.

DAT-expressing dopaminergic neurons are found in other brain regions, including the hypothalamus and dopaminergic neurons innervate brain regions other than the striatum, most notably, the prefrontal cortex. Though levels of DAT in the prefrontal cortex have been shown to be relatively low, stress can increase expression, causing hypoactivity of DA in this structure (Novick et al., 2015). Because our DREADD targets all neurons that contain this transporter, inactivation of neurons containing DAT in the mPFC could have some effects on behavior and cognition. Furthermore, inhibition of VTA neurons through DREADD mediated silencing could have some effects on reward sensitivity. For example, projections from the VTA to the mPFC have been implicated in conditioned place preference, self-administration, and other paradigms associated with reward (Han, Jing, Zhao, Wu, Song, & Li, 2017); notably however, the inhibition of midbrain DA neurons did not seem to have effects on consummatory and effortful responding in this study.

A2A receptors are located, albeit at low levels, in the neocortex, hippocampus and olfactory bulb (Vaughn, Brown, McCoy, & Kuhar, 1996; Sheth et al., 2014). A2A receptors have also been found in microglia and astrocytes which have been implicated in disorders such as Alzheimer's disease. In one study, ablation of A2A receptors located on astrocytes improved spatial memory (Orr et al., 2015). Similar results were also found in blockade of A2A hippocampal receptors, which allowed for decreases in synaptotoxicity mediated memory loss (Canas et al., 2009). Thus, A2A-directed expression of DREADDs in neurons other than striatopallidal cells could contribute to our results. Future assays will also include immunofluorescence in order to see exactly where the DREADDs in this study are being expressed, which will allow for a better understanding of which structures are being affected by CNO treatment.

While CNO has been shown to be effective in their action on DREADD receptors, location and levels of expression across the brain must also be taken into account. This can be assessed through immunofluorescence, as the DREADD construct used in this study contains both an HA and mCitrine tag. Through the use of specific antibodies, this will allow us to find exactly where DREADD receptors are being expressed. This will also give us insight into which structures show the highest receptor density. One caveat to the use of immunofluorescence is that oftentimes expression levels are too small to visualize (Smith, Bucci, Luikart, & Mahler, 2016). Another way location of DREADD receptors can be visualized is through μ PET scans. In these studies, CNO was injected into DREADD positive animals before being placed in the scanner, thus allowing for visualization and measurement of activity in brain regions of interest (Michaelides et al., 2013). Though this can be expensive, it is another possible way of understanding the

biological effects of DREADDs (Whissell, Tohyama, Martin, 2016). Thus, combining some of these assays will allow us to measure function and localization of DREADD receptors in the double transgenic mice that were tested in the current study. Something that, to our knowledge, has not yet been carried out.

Another limitation is that biological efficacy of the DREADDs have not been specifically confirmed in our transgenic models. Although this is a limitation, there are several ways in which we are able to accomplish this. Several groups have undertaken electrophysiological studies in order to analyze the strength and temporal activation of DREADD receptors *in vivo* (Smith, Bucci, Luikart, & Mahler, 2016). For example, one study found that CNO caused a decrease in neuronal firing in 79% of cells, suggesting an effect of CNO on a majority of cells of interest (Mahler, Vazey, & Aston-Jones, 2013). Other than electrophysiology, immunohistochemistry of *fos* allows for measurement of neural activity in populations of interest (Krashes et al., 2011). Similarly, microdialysis can be used in order to measure levels of DA release in structures of interest (Chefer, Thompson, Zapata, & Shippenberg, 2009). This allows for quantification of the effect each DREADD manipulation has on endogenous signalling.

Future Aims

A future experiment from this study is to use an A2A-Gq mouse in order to see if the results found in the current study are able to be reversed. While A2A/GiDREADD mice in the current study allowed for striatopallidal neurons to be silenced, A2A/GqDREADD mice would have the opposite effect, allowing for greater excitation of these neurons. These mice have been used before, showing that GiDREADD activation resulted in increases in movement while GqDREADD activation resulted in

the opposite effect (Zhu, Ottenheimer, & DiLeone, 2016). While this differentiates from the results reported here, it would be worthwhile to inspect whether these results persist across different manipulations.

Another direction is to allow mice to choose between a less salient food reward that requires no effort versus a more salient reward that requires a higher level of effort to obtain. This is a successful measurement of cost/benefit analyses made popular by the Salamone lab (Salamone & Correa, 2002; Salamone et al., 1996). This experiment often shows that DA depleted rats will choose a free, less palatable reward while control rats will work for the more salient reward. This would be interesting, as A2A/DREADD mice showed deficits in both voluntary and effort-based consumption of a palatable reward.

Conclusion

In sum, double transgenic mice in the current study responded to CNO in different, but significant ways. DAT/DREADD mice showed reductions in locomotion after treatment with a DREADD agonist, however these effects did not carry over to voluntary or effort-based consumption of sweetened condensed milk. Oppositely, A2A/DREADD mice showed stable locomotion across saline and low doses of CNO, while also showing reductions in consumption of SCM regardless of the effort involved. This study shows evidence that the roles of pre- and postsynaptic DA transmission can be dissociated, as inhibition of either population was associated with varying behavioral effects. Thus, according to the results reported here, presynaptic release is not required for consumption of a salient natural reward, whether effort to gain the reward is required or not. Oppositely, D2 receptor mediated inhibition of striatopallidal neurons does not

have an effect on ambulation but is integral in regards to motivation and consummatory behavior.

Total distance traveled after saline treatment

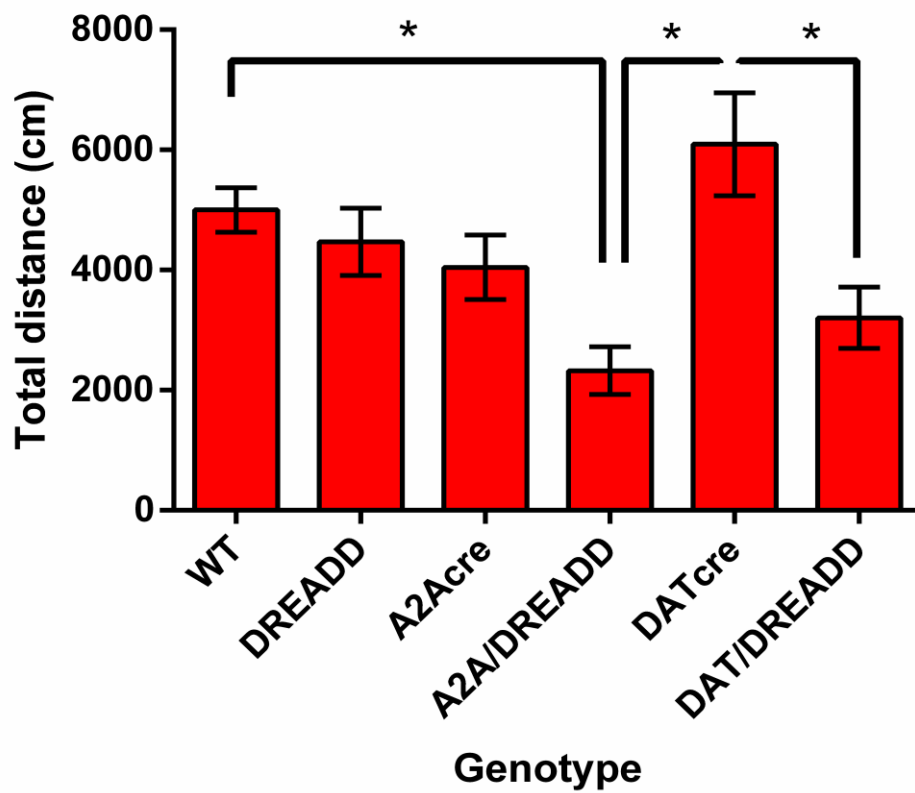


Figure 1: Total distance traveled after treatment with saline. A2A/DREADD mice had significantly less ambulation than both wildtype and DATcre animals. DAT/DREADD also traveled a significantly shorter distance than DATcre mice.

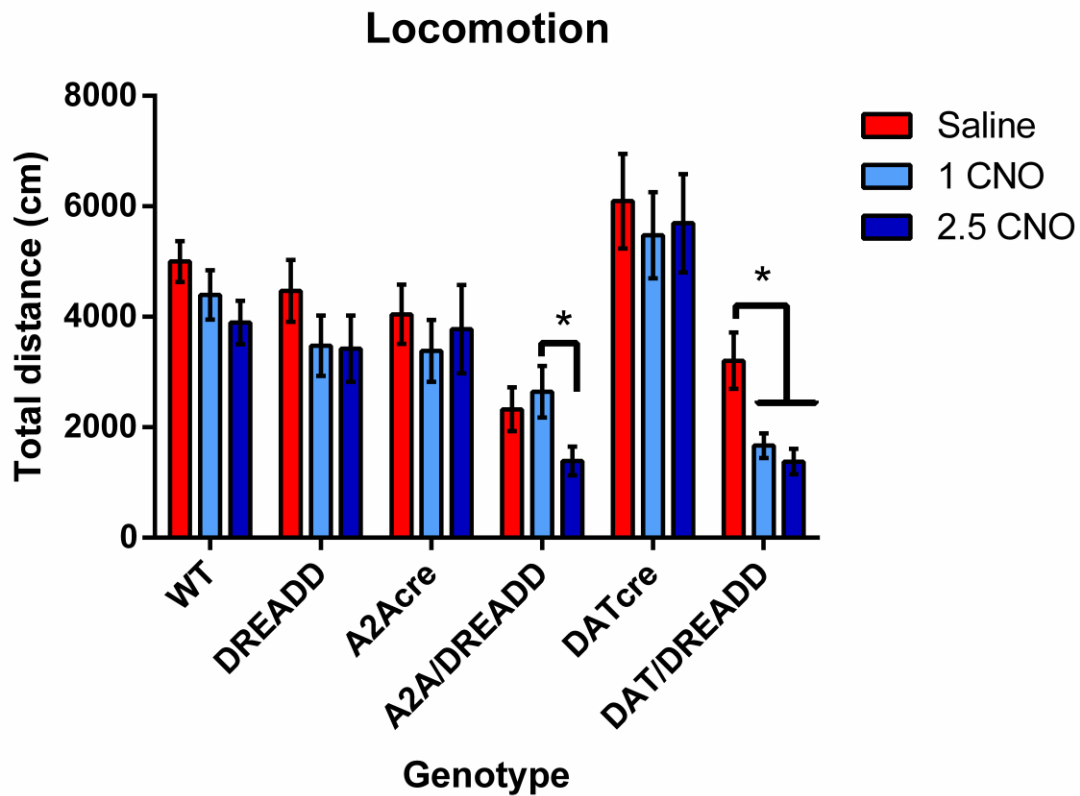


Figure 2: Total distance traveled across all treatments and genotypes. A2A/DREADD mice traveled a larger distance when given 1mg/kg CNO compared to 2.5mg/kg CNO. Furthermore, DAT/DREADD mice moved significantly less when treated with both doses of CNO in contrast to saline.

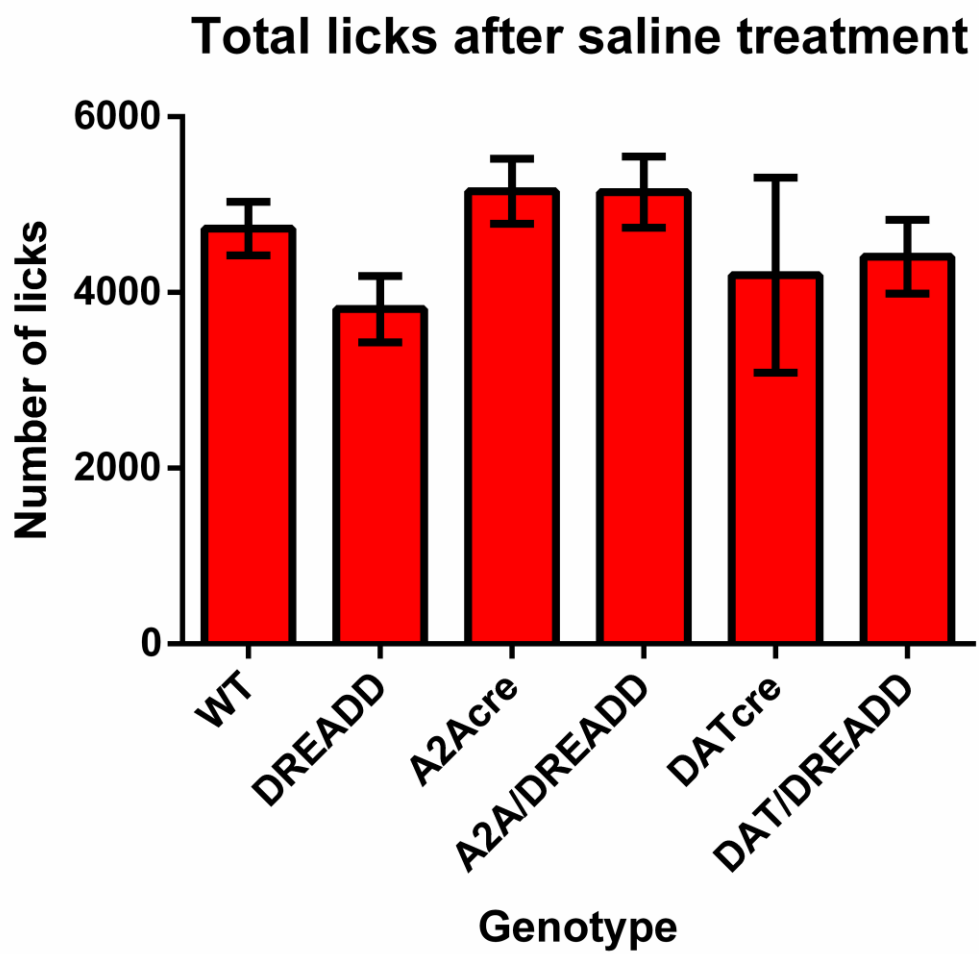


Figure 3: Total number of licks after treatment with saline. No significant effects were found.

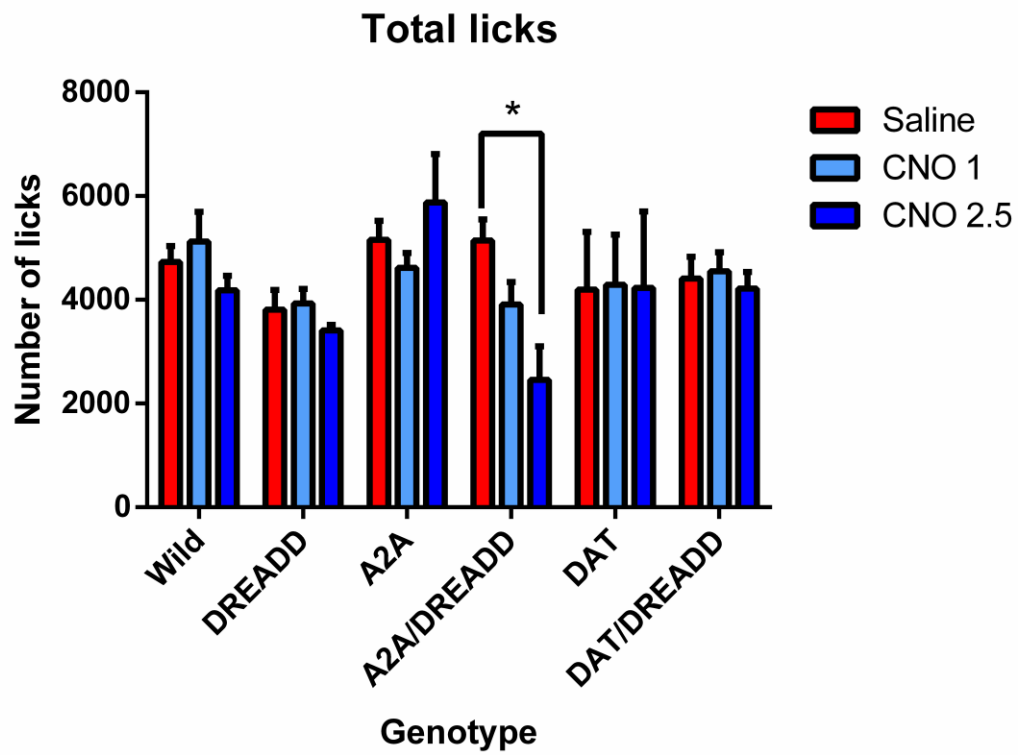


Figure 4: Total number of licks across all genotypes and treatments. A2A/DREADD mice registered a significant decrease in licks after treatment with 2.5mg/kg CNO in comparison to saline.

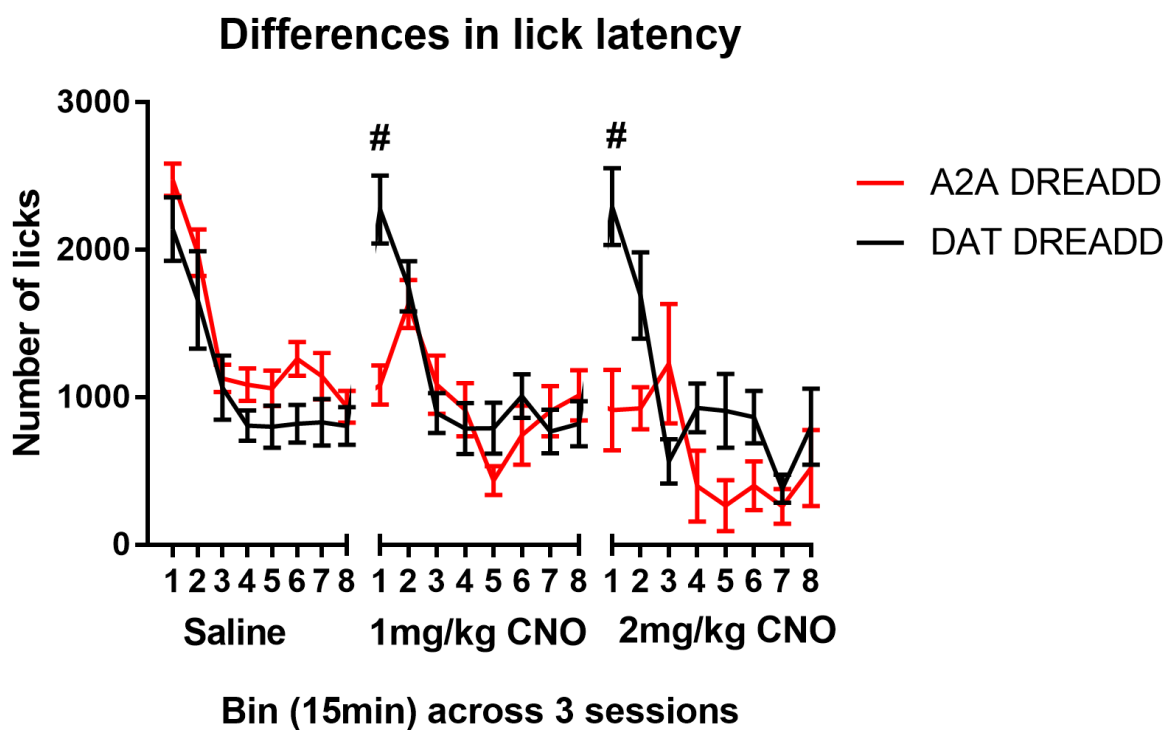


Figure 5: Total licks summed across three sessions. CNO treated A2A/DREADD mice showed significant deficits in number of licks for the first 15-min in comparison to wildtype, DAT/DREADD, and A2Acre mice. Only DAT/DREADD mice are plotted, as it is representative of the other two effects listed. Also, A2A/DREADD registered fewer licks when treated with both doses of CNO in contrast to saline.

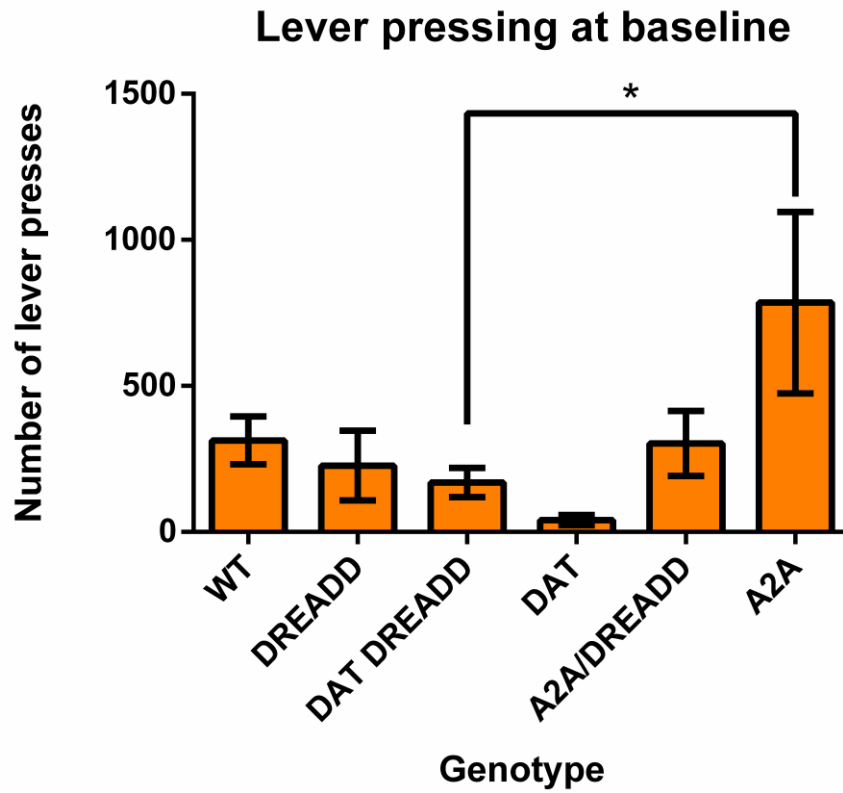


Figure 6: Total number of baseline presses in all genotypes. A2A mice registered significantly more lever presses in comparison to DAT/DREADD.

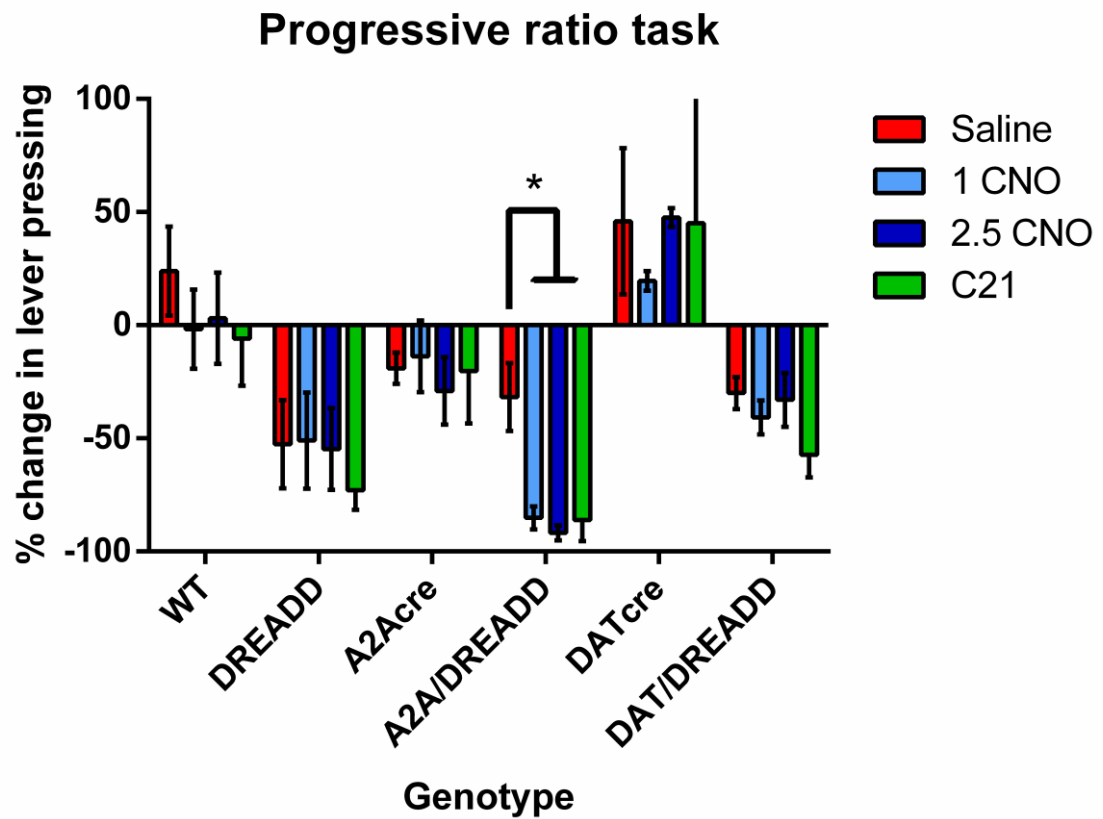


Figure 7: % change from baseline lever pressing across all treatments and genotypes. In contrast to baseline responding, A2A/DREADD mice showed a significant decrease in lever pressing for all doses of CNO and C21.

Genotype	n
A2Acre	14
A2A/DREADD	21
DATcre	18
DAT/DREADD	24
DREADD	19
Wildtype	32

Figure 8: Sample used for analysis of locomotor activity

Genotype	Saline	1mg/kg CNO	2.5mg/kg CNO
A2Acre	14	14	4
A2A/DREADD	19	19	10
DATcre	3	3	3
DAT/DREADD	18	18	13
DREADD	4	4	4
Wildtype	18	18	8

Figure 9: Sample used for analyses of voluntary consumption

Genotype	n
A2Acre	4
A2A/DREADD	9
DATcre	2
DAT/DREADD	12
DREADD	3
Wildtype	8

Figure 10: Sample used for analyses of the progressive ratio task

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