Targeting Dopamine D$_2$, Adenosine A$_{2A}$, and Glutamate mGlu$_5$ Receptors to Reduce Repetitive Behaviors in Deer Mice

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ABSTRACT

Repetitive behaviors are seemingly purposeless patterns of behavior that vary little in form and are characteristic of many neurodevelopmental, psychiatric, and neurologic disorders. Our work has identified an association between hypofunctioning of the indirect basal ganglia pathway and the expression of repetitive behavior in the deer mouse model. In this study, we targeted indirect pathway cells of the striatum with single drugs and drug combinations that bind to dopamine D$_2$, adenosine A$_{2A}$, and glutamate mGlu$_5$ receptors. These receptors function both individually and as receptor heteromers. We found that only the triple drug cocktail (L-741,626+CGS21680+CDPPB) that was designed to increase striatal indirect basal ganglia pathway cell function reduced repetitive behavior in adult male deer mice. No single drug or double drug combinations were effective at selectively reducing repetitive behavior. We found this triple drug cocktail reduced repetitive behavior in both short-acting and long-acting formulations and was effective throughout 7 days of daily administration. Conversely, another triple drug cocktail (quinpirole+SCH58261+MTEP) that was designed to further reduce striatal indirect basal ganglia pathway cell function caused a significant increase in repetitive behavior. Significant and behaviorally selective effects on repetitive behavior were only achieved with the triple drug cocktails that included doses of L-741,626 and quinpirole that have off-target effects (e.g., dopamine D$_3$ receptors). These data further a role for decreased indirect basal ganglia pathway activation in repetitive behavior and suggest that targeting these receptors and/or heteromeric complexes on the indirect pathway neurons of the striatum may offer pharmacotherapeutic benefit for individuals with repetitive behavior disorders.

Introduction

Repetitive behaviors are present in a number of neurodevelopmental, neurologic, and psychiatric disorders (e.g., autism spectrum disorder, obsessive-compulsive disorder, frontotemporal dementia). These behaviors exhibit little variation in form and are without obvious function. In neurodevelopmental disorders specifically, there is a wide array of repetitive behaviors, ranging from stereotypies to compulsions (Bodfish et al., 2000). Repetitive behaviors have a negative impact on the individual and his or her family. The presence of these rigid and inflexible behaviors can impede treatment of other phenotypic traits of the disorder (Green et al., 2006b; Cunningham and Schreibman, 2008), become the genesis of mood and other behavioral problems (Green et al., 2006b), and are a source of parental stress (Bishop et al.,

2007). Unfortunately, we have no effective pharmacological treatments for these maladaptive behaviors. Finding treatments that specifically target the neural pathways that mediate repetitive behavior is the best strategy for developing effective pharmacotherapy (Lacivita et al., 2017).

The wide range of repetitive behavior phenotypes and the spectrum of disorders associated with repetitive behavior suggest that the specific molecular mechanisms that mediate repetitive behavior may vary among individuals and across clinical disorders. This implies that the common dysfunction across affected individuals is altered output of neural circuitry. Basal ganglia circuitry is predominantly associated with repetitive behavior (Muehlmann and Lewis, 2012) and is made up of the direct and indirect pathways, which are parallel and complementary in function (reviewed in Gerfen and Surmeier (2011)). Proper, adaptive expression of basal ganglia-mediated behaviors depends on the appropriate balance of activity from these two pathways (Bateup et al., 2010). Activation of the direct pathway leads to selection of desired motor programs and locomotor behavior, whereas activation of the indirect pathway leads to behavioral inhibition (Freeze et al., 2013; Meng et al., 2018). The population of spiny projection neurons of the striatum, the input region of basal

ABBREVIATIONS: CDPPB, 3-Cyano-*N*-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide; CGS21680, 3-{4-[2-({6-Amino-9-[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxytetrahydro-2-furanyl]-9H-purin-2-yl}amino)ethyl]phenyl}propanoic acid; L-741,626, 4-(4-Chlorophenyl)-1-(1H-indol-3-ylmethyl)-4-piperidinol; MTEP, 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine hydrochloride; NAM, negative allosteric modulator; PAM, positive allosteric modulator; Quinpirole, (4aR-*trans*)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline hydrochloride; RM-ANOVA, repeated measures analysis of variance; SCH58261, 7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine; STN, subthalamic nucleus.

ganglia circuitry, is equally divided between direct and indirect pathway neurons (Matamales et al., 2009).

Animal models provide an important means for identifying the neural circuitry of repetitive behavior. Our laboratory uses outbred deer mice that exhibit spontaneous repetitive behavior because of typical, yet impoverished, laboratory housing. Deer mice, when housed in standard laboratory cages, exhibit high rates of repetitive behavior consisting of vertical jumping and/or backward somersaulting (Muehlmann et al., 2015). Several lines of evidence from our group suggest that this repetitive behavior is a result of indirect basal ganglia pathway hypoactivation. For instance, in the subthalamic nucleus (STN), a relay region within the indirect basal ganglia pathway, less neuronal activation was observed in deer mice with high rates of repetitive behavior compared with deer mice with low rates of repetitive behavior (Tanimura et al., 2011). Our laboratory has also shown that housing deer mice in an enriched environment significantly reduces repetitive behavior, an outcome associated with increased neuronal activation and dendritic spine density in the STN (Bechard et al., 2016). This suggests decreased indirect pathway function is involved in deer mouse repetitive behavior and is consistent with the role of STN in behavioral inhibition (Fife et al., 2017).

Indirect pathway neurons of the striatum express neurotransmitter receptors that form heteromeric complexes [for review see Ferre et al. (2007), Borroto-Escuela et al. (2016)]. One type of heteromeric complex consists of the dopamine D_2 receptor, the adenosine A_{2A} receptor, and the glutamate $mGlu_5$ receptor (Fuxe et al., 2003; Cabello et al., 2009). A_{2A}-and $mGlu_5$-receptor agonism synergistically reduces dopamine binding at the D_2 receptor (Ferre et al., 1999; Rimondini et al., 1999; Popoli et al., 2001) and increases neurotransmitter release from striatal indirect pathway neurons (Diaz-Cabiale et al., 2002). Costimulation of A_{2A} and $mGlu_5$ receptors also synergistically activates cell signaling cascades and immediate early genes in the striatum (Ferre et al., 2002; Fuxe et al., 2003; Nishi et al., 2003; Schiffmann et al., 2007; Dell'anno et al., 2013). In the present studies, we tested the hypotheses that indirect basal ganglia pathway activation would reduce repetitive behavior, and inactivation would increase repetitive behavior (Fig. 1). A pharmacological strategy was used to test these hypotheses. Drug cocktails targeting these dopamine D_2-, adenosine A_{2A}-, and glutamate $mGlu_5$-receptor heteromers were tested. We designed two different drug cocktails, one to reduce repetitive behavior and one to increase repetitive behavior. For the triple drug cocktail designed to reduce repetitive behavior, we also used peanut oil as a vehicle to provide a depot preparation for a longer duration of action. We then tested the effects of subchronic administration (daily for 7 days) of this depot preparation.

Materials and Methods

Experiment 1: Acute Administration of a Drug or Drug Combinations to *Reduce* Repetitive Behavior

Animals. One hundred and twelve adult male deer mice (*Peromyscus maniculatus*) were used in experiment 1. They were acquired from our established breeding colony, weaned at 21 days of age, and housed four to seven mice per standard cage ($29 \times 18 \times 13$ cm). Room temperature was maintained within a range of 70°–75°F, and a 16:8 light/dark cycle, with lights off at 10:00 AM. Food and water were available ad libitum and two Nestlet squares were provided for nest construction. All procedures were performed in accordance with the guidelines set forth in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the University of Florida Institutional Animal Care and Use Committee.

Drugs. The dopamine D_2 receptor antagonist, L-741,626, was purchased from Tocris Bioscience (Bio-Techne Corporation, Minneapolis, MN) and suspended in 25% dimethyl sulfoxide (DMSO; Thermo Fisher Scientific, Waltham, MA) in saline at either 0.3, 1, or 3 mg/ml
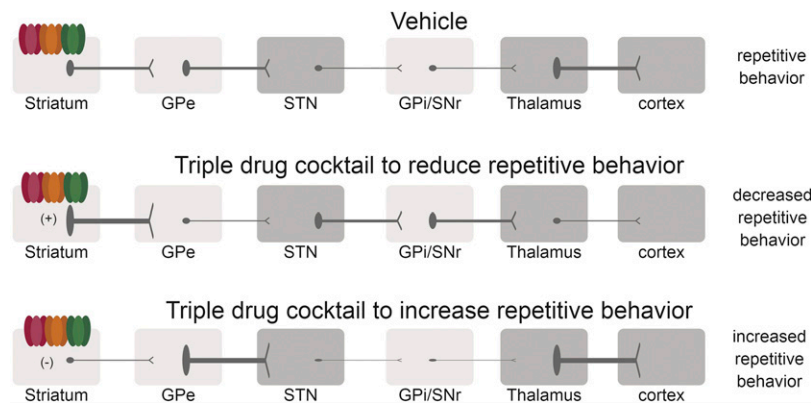

Fig. 1. (A) Schematic of the indirect basal ganglia pathway in deer mice. Our previous work identified lower neuronal activation in the STN in deer mice with high rates of repetitive behavior. We hypothesize that this reduced function of the STN leads to less inhibitory output of the indirect basal ganglia pathway and disinhibition of the thalamus, which leads to excitation of the primary motor cortex and to the expression of repetitive behavior. (B) Schematic of hypothesized changes in the indirect basal ganglia pathway after administration of the triple drug cocktail of a dopamine D_2 receptor antagonist, adenosine A_{2A} receptor agonist, and glutamate $mGlu_5$ receptor positive allosteric modulator. These receptors are located on the indirect pathway neurons in the striatum. We hypothesize that this triple drug cocktail increases GABA release from striatopallidal neurons, leading to increased STN neuronal activation and ultimately reduced cortical activation and reduced repetitive behavior. (C) Schematic of hypothesized changes in the indirect basal ganglia pathway after administration of the triple drug cocktail of a dopamine D_2 receptor agonist, adenosine A_{2A} receptor antagonist, and glutamate $mGlu_5$ receptor negative allosteric modulator. We hypothesize that this triple drug cocktail decreases GABA release from striatopallidal neurons, leading to a further reduction in STN neuronal activation and ultimately increased cortical activation and increased repetitive behavior. GABAergic nuclei are shown in light gray boxes; glutamatergic nuclei are shown in dark gray boxes. The size of the projection neurons relates to the hypothesized direction of neuronal activation changes, such that thinner projections correspond to reduced neuronal activation and thicker projections correspond to increased neuronal activation, relative to the vehicle group. GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; SNr, substantia nigra pars reticulata.

and injected at either 3, 10, or 30 mg/kg, respectively. The solution was sonicated and vortexed repeatedly up until time of injection. The adenosine A_{2A} agonist, CGS21680, and the glutamate $mGlu_5$ positive allosteric modulator (PAM), CDPPB, were each acquired through the National Institute of Mental Health's Chemical Synthesis and Drug Supply Program. CGS21680 was dissolved in 25% DMSO in saline at 0.005 mg/ml and injected at 0.05 mg/kg. CDPPB was suspended in 25% DMSO in saline at either 0.3 or 3 mg/ml and injected at 3 or 30 mg/kg, respectively. This solution also required repeated sonication and vortexing up until time of injection. L-741,626 and CDPPB required 25% DMSO to reach a suitable suspension. This is well below 32% DMSO, which has been shown to decrease locomotor activity (Castro et al., 1995) and although some signs of discomfort were observed, these signs resolved within the first minute following injection.

Dose Selection and Drug Treatments. Single-drug dose response analyses were conducted to evaluate the efficacy of the dopamine D_2 receptor antagonist, L-741,626, and the glutamate $mGlu_5$ PAM, CDPPB, to reduce repetitive behavior in deer mice. These drugs were chosen for their receptor selectivity on the basis of published in vitro work (Jarvis et al., 1989; Kinney et al., 2005; Grundt et al., 2007). Furthermore, the drug dose ranges were chosen on the basis of published behavioral studies (Millan et al., 2004; Kinney et al., 2005). The D_2 receptor antagonist used, L-741,626, has affinity at the dopamine D_3 and D_4 receptors, though binding is at concentrations at least 15- to 40-fold higher than at the D_2 receptor (Kulagowski et al., 1996; Grundt et al., 2007). We did not measure drug bioavailability or brain levels of these drugs following subcutaneous administration, so D_2 selectivity cannot be confirmed. CDPPB, at all concentrations used in these experiments, does not reach micromolar brain concentrations and has no submicromolar activity at any of the >175 receptors, transporters, ion channels, or enzymes that have been tested (Kinney et al., 2005; Feng et al., 2015). We found previously that the 0.05 mg/kg dose of CGS21680, the adenosine A_{2A} receptor agonist, did not reduce repetitive behavior in deer mice and that higher doses resulted in nonselective motor suppression (Tanimura et al., 2010a). CGS21680 has been shown to also bind to adenosine A_1 receptors at a 140-fold higher concentration than for A_{2A} receptors (Hutchison et al., 1989). Information regarding the hepatic metabolism of these compounds has not be reported, though L-741,626 significantly inhibits the gene expression of many cytochrome P450 enzymes including CYP1A1, CYP1A2, CYP1B1, CYP2C11, CYP2D1, CYP2D2, CYP2E1, CYP3A1, and CYP3A2 (Daskalopoulos et al., 2012; Harkitis et al., 2015).

We injected separate cohorts of mice at the following doses: 3 mg/kg L-741,626 (cohort 1-1; $n = 13$), 10 mg/kg L-741,626 (cohort 1-2; $n = 9$), 30 mg/kg L-741,626 (cohort 1-3; $n = 13$), and 3 and 30 mg/kg CDPPB (cohort 1-4; $n = 11$). We also tested a single dose of CGS21680 (0.05 mg/kg, cohort 1-5; $n = 12$).

Our investigations of double drug combinations were also run in separate cohorts of mice. We selected the highest dose of the individual drugs that were run in the single drug experiments: L-741,626 at 30 mg/kg, CGS21680 at 0.05 mg/kg, and CDPPB at 30 mg/kg. We injected separate cohorts of mice with the following double drug combinations: L-741,626 + CGS21680 (cohort 1-6; $n = 12$), CGS21680 + CDPPB (cohort 1-7; $n = 13$), L-741,626 + CDPPB (cohort 1-8; $n = 12$). Finally, a separate cohort of deer mice received the triple drug cocktail at the individual doses used in the double drug combinations (cohort 1-9; $n = 12$). When drugs were given together as double drug combinations or the triple drug cocktail, a single solution was made and administered in a single injection.

Repetitive Behavior Testing. The testing protocol involved removing mice from their home cages, weighing them, and placing them singly in standard testing cages (22 × 15 × 28 cm) 1 hour prior to the beginning of the dark cycle to allow for habituation. Food and water were provided. Each animal was assessed for the 8 hours of the dark cycle.

The repetitive behavior observed in deer mice consists largely of two response topographies: jumping and backward somersaulting. The former topography involves the deer mouse rearing against the cage wall and engaging in vertical hindlimb jumping. The second topography involves the deer mouse rotating its body such that it starts with all four paws on the cage floor, inverts its ventral surface to the cage top, and returns to the cage floor, upright and on all four paws. As these behaviors involve vertical activity, they were quantified using photobeam arrays which, when interrupted, recorded a count (Columbus Instruments, Columbus, OH). We termed these "repetitive behavior counts" and calculated these repetitive behavior counts for the 1 hour preceding injection and the 1 hour following injection.

We also employed a video surveillance system that allows digital recording of each automated test cage during the entire 8 hours dark cycle. This permitted precise determination of the individual behavior of the animals and precise estimates of the reliability of the automated apparatus.

All of these studies were run in a random order crossover design, wherein each mouse was also administered a vehicle injection (25% DMSO in saline) on a test day separated by at least a week from the drug challenge. All injections were administered subcutaneously at 4:00 PM (6 hours following lights off), a time when repetitive behavior counts are high in deer mice (Tanimura et al., 2010b), leaving 2 hours of the dark cycle remaining to study the effects of the drugs on repetitive behavior.

Locomotor Monitoring. To assess the selectivity of the motor effects of the triple drug cocktail, we injected five male deer mice with the triple drug cocktail (L-741,626 at 30 mg/kg, CGS21680 at 0.05 mg/kg, and CDPPB at 30 mg/kg) or 25% DMSO vehicle in a random order crossover design. The two tests of locomotor activity were 3 days apart. We used the VersaMax Animal Activity Monitoring System (Accuscan Instruments, Inc., Columbus, OH) to measure total distance traveled (in centimeters). Mice were left to habituate to the locomotor monitors (40 × 40 × 30.5 cm) for 10 minutes and then injected with either the triple drug cocktail or DMSO vehicle. Locomotion was tested in individual mice immediately following injection, and testing lasted 1 hour. Total distance traveled during that hour was calculated.

Data Analysis. For each of the 107 deer mice in the repetitive behavior testing portion of experiment 1, we totaled the number of repetitive behavior counts that were recorded in the hour before injection and the hour following injection on the day they received vehicle and the day(s) they received drug. The dependent measure used for this experiment was a difference score of the total number of repetitive behavior counts the 1 hour following injection minus the total number of repetitive behavior counts in the 1 hour before injection. This within-subject analysis was used to control for rate of repetitive behavior exhibited by any given deer mouse on any given day, which can be highly variable. We expected that when mice were administered vehicle their difference scores would be near zero or slightly positive, which corresponds to no change or slight increases in the rate of repetitive behavior at this time during their dark cycle. The basis of this expectation was our measurement of repetitive behavior in deer mice across the dark cycle (Tanimura et al., 2010b) that showed repetitive behavior counts approach asymptotic levels around 4:00 PM. Likewise, a negative difference score indicates a reduction in the number of repetitive behaviors postinjection relative to preinjection.

Within each cohort, the primary analysis used was a paired t test [or repeated measures analysis of variance (ANOVA) for cohort 1-4] to compare the difference scores (post- minus preinjection) on the day the mice received vehicle and the difference scores (post- minus preinjection) on the day the mice received drug. For any drug(s) that met the significance threshold ($\alpha = 0.05$), we performed a secondary analysis, which compared the difference score from that cohort on the day they received drug compared with the difference scores from all other mice in the experiment on the day they received vehicle, using an unpaired t test. This was to confirm that the result of the primary analysis was not spurious or the result of a high vehicle difference score in that particular cohort. We further confirmed all significant drug effects by

using a paired t test on only postinjection counts (during the 60 minutes following injection) within that cohort. For visual representation of the data, we present 1-hour-preinjection and 1-hour-postinjection repetitive behavior totals instead of the difference scores. Data from the locomotor monitoring portion of experiment 1 were compared using a paired, two-tailed t test with an alpha level of 0.05.

Experiment 2: Acute Administration of a Drug or Drug Combinations to *Increase* Repetitive Behavior

Animals. Forty four adult male deer mice were used in this experiment to evaluate the effects on repetitive behavior by single drugs, double drug combinations, and a triple drug cocktail made up of a dopamine D_2 receptor agonist, an adenosine A_{2A} antagonist, and/or a glutamate $mGlu_5$ negative allosteric modulator (NAM). Mice were weaned and housed as described in experiment 1.

Drugs. Quinpirole hydrochloride (a dopamine D_2 receptor agonist), SCH58261 (an adenosine A_{2A} receptor antagonist), and MTEP hydrochloride (a glutamate $mGlu_5$ receptor NAM) were purchased from Sigma-Aldrich (St. Louis, MO). They were dissolved in 10% DMSO and saline at 0.3 mg/ml for quinpirole, 0.1 mg/ml for SCH58261, and 0.5 mg/ml for MTEP.

Drug Treatments. Single-drug analyses were conducted to evaluate the efficacy of the dopamine D_2 receptor agonist, quinpirole, the adenosine A_{2A} receptor antagonist, SCH58261, and the glutamate $mGlu_5$ receptor NAM, MTEP, to increase repetitive behavior in deer mice. We injected separate cohorts of mice at the following doses: 3 mg/kg for quinpirole (cohort 2-1; $n = 6$), 5 mg/kg MTEP (cohort 2-2; $n = 6$), or 1 mg/kg SCH58261 (cohort 2-3; $n = 7$). Doses were chosen on the basis of previous behavioral studies (Hsu et al., 2010; Luque-Rojas et al., 2013; Ribeiro et al., 2014).

Our investigations of double drug combinations were also run in separate cohorts of mice using the doses of drug used in each of the single drug cohorts. These cohorts received either quinpirole + SCH58261 (cohort 2-4; $n = 6$), SCH58261 + MTEP (cohort 2-5; $n = 6$), or quinpirole + MTEP (cohort 2-6; $n = 6$). Finally, a separate cohort of deer mice received a triple cocktail of drugs at the individual doses used in the double drug combinations (cohort 2-7; $n = 7$). When drugs were given together as double drug combinations or the triple drug cocktail, a single solution was made and administered in a single injection.

Repetitive Behavior Testing. Repetitive behavior was quantified as described in experiment 1. All of these studies were run in a random order crossover design, wherein each mouse was also administered a vehicle injection (10% DMSO in saline) on a test day separated by at least a week from the drug challenge. For this experiment, we hypothesized that the triple drug cocktail would increase the expression of repetitive behavior. We performed all injections subcutaneously and at a time during the dark cycle when repetitive behavior counts were low (2:00 PM; Tanimura et al., 2010b) to be able to detect increases in repetitive behavior, which may have been masked if drugs had been given at a time in the day when repetitive behavior counts were high (i.e., 4:00 PM).

Data Analysis. We totaled the number of repetitive behavior counts that were recorded in the 30 minutes before injection and the 30 minutes following injection on the day the deer mice received vehicle and the day they received drug. A 30-minute cut-off was used on the basis of the rapid clearance and short half-life of MTEP in mice (Green et al., 2006a). To determine drug effects on repetitive behavior, we again used the postinjection minus preinjection difference scores. We expected that when mice were administered vehicle their difference scores would be near zero or slightly negative, which corresponds to no change or slight decreases in the rate of repetitive behavior at this time during their dark cycle. The basis of this expectation was our measurement of repetitive behavior in deer mice across the dark cycle (Tanimura et al., 2010b) that showed repetitive behavior counts were low around 2:00 PM. Likewise, a positive difference score indicates an

increase in the number of repetitive behaviors postinjection relative to preinjection.

As in experiment 1, the primary analysis used was a paired t test to compare the difference scores (post- minus preinjection) on the day the mice received vehicle and the difference scores (post- minus preinjection) on the day the mice received drug for each separate cohort. For any drug(s) that met the significance threshold ($\alpha = 0.05$), we performed a secondary analysis, which compared the difference score from the cohort on the day they received drug compared with the difference scores from all other mice in the experiment on the day they received vehicle, using an unpaired t test. This was to confirm that the result of the primary analysis was not spurious or owing to a low vehicle difference score in that particular cohort. We further confirmed all significant drug effects by using a paired t test on only postinjection counts (during the 30 minutes following injection) within that cohort. For visual representation of the data, we present 30-minute preinjection and 30-minute postinjection repetitive behavior totals instead of the difference scores.

Experiment 3: Acute Administration of Depot Drug or Drug Combinations to *Reduce* Repetitive Behavior

Animals. Ninety-nine adult male deer mice were used in this experiment to evaluate an oil-based formulation of the single drugs, double drug combinations, and the triple drug cocktail evaluated in experiment 1 (using a dopamine D_2 receptor antagonist, an adenosine A_{2A} agonist, and a glutamate $mGlu_5$ PAM). Mice were weaned and housed as described in experiment 1.

Drugs. L-741,626, CGS21680, and CDPPB were acquired as described in experiment 1. Each drug was suspended in peanut oil (Sigma-Aldrich) and stirred for at least 1 hour before injection. No DMSO was required for drugs to reach suspension in peanut oil. L-741,626 was suspended at 0.5, 0.75, 1.5, or 3.0 mg/ml, CGS21680 was suspended at 0.005 and 0.03 mg/ml, and CDPPB was suspended at 1.5 or 3.0 mg/ml. When drugs were given together as double combinations or the triple drug cocktail, a single solution was made and administered in a single injection. All injections were administered subcutaneously.

Drug Treatments. On the basis of the known hydrophilicity of each drug, additional testing of efficacy and nonselective motor suppression was required. Using separate cohorts of mice we tested L-741,626 at 30 mg/kg (cohort 3-1; $n = 13$), 15 mg/kg (cohort 3-4; $n = 11$), 7.5 mg/kg (cohort 3-5; $n = 9$), and 5 mg/kg (cohort 3-6; $n = 7$). CGS21680 was tested at 0.05 mg/kg (cohort 3-2; $n = 8$) and 0.3 mg/kg (cohort 3-7; $n = 11$). CDPPB was tested at 30 mg/kg (cohort 3-3; $n = 13$) and 15 mg/kg (also in cohort 3-4; $n = 11$). In addition, we ran a crossover experiment comparing the oil vehicle and double drug combinations, L-741,626 (5 mg/kg) + CGS21680 (0.3 mg/kg), L-741,626 (5 mg/kg) + CDPPB (15 mg/kg), and CGS21680 (0.3 mg/kg) + CDPPB (15 mg/kg) in a separate cohort of mice (cohort 3-8; $n = 10$). We also ran a comparison of the triple drug cocktail (using the drug doses used in the double drug combination comparisons) and oil vehicle in a separate cohort of mice (cohort 3-9; $n = 11$).

Repetitive Behavior Testing. Repetitive behavior was quantified as described in experiment 1. All of these studies were run in a random-order crossover design, wherein each mouse was also administered a vehicle injection (peanut oil) on a test day separated by at least a week from the drug challenge. For the single and double drug analyses, injections were administered at 2:00 PM to evaluate the duration of efficacy of any drugs or drug combinations. For the triple drug cocktail assessment, injections were given as soon as the lights turned off (10:00 AM) to evaluate the full duration of efficacy.

Locomotor Monitoring. To assess the selectivity of the motor effects of the triple drug cocktail, we injected six male deer mice with L-741,626 (5 mg/kg), CGS21680 (0.3 mg/kg), and CDPPB (15 mg/kg) or peanut oil vehicle in a random-order, crossover design. The two tests of locomotor activity were 7 days apart. We used the VersaMax Animal Activity Monitoring System described in experiment 1.

Data Analysis. We did not use difference scores in this experiment because the duration of action of the peanut oil preparation was longer, which allowed for a more accurate assessment of the repetitive behavior in the deer mice without the need to control for baseline values. The dependent measure used for this experiment was the total number of repetitive behavior counts that were recorded during the 4 hours following injection. For these analyses, we used paired, two-tailed t tests. For visual representation of the triple drug cocktail data, we present repetitive behavior counts for each hour of the 8-hour test. Data from the locomotor monitoring portion of experiment 3 were compared using a paired, two-tailed t test with an alpha level of 0.05.

Experiment 4: Subchronic Administration of Depot Triple Drug Cocktail to *Reduce* Repetitive Behavior

Animals. Twenty adult male deer mice were randomly assigned to either the triple drug cocktail group ($n = 10$) or the peanut oil vehicle group ($n = 10$). Mice were weaned and housed as described in experiment 1.

Drugs. Drugs were acquired, prepared, and injected as described for experiment 3.

Drug Treatments. Independent groups of deer mice were injected with either the triple drug cocktail (L-741,626 at 5 mg/kg, CGS21680 at 0.3 mg/kg, and CDPPB at 15 mg/kg) or peanut oil vehicle. Injections were administered subcutaneously at lights off (10:00 AM) each day for 7 days.

Repetitive Behavior Testing. Repetitive behavior testing was conducted as described in experiment 1 but only on days 1, 4, and 7 of drug or vehicle administration. On days 2, 3, 5, and 6 of drug or vehicle administration, each mouse was weighed, injected, and then immediately returned to their home cage for the rest of the day. Food pellets were weighed and water bottle volumes were noted before and after the 8-hour repetitive behavior testing session to measure food and water consumption on the repetitive behavior test days (i.e., days 1, 4, and 7).

Data Analysis. Total repetitive behavior counts for the 8-hour test, as well as body weight, food consumption, and water consumption were compared between groups using a two-way repeated measures analysis of variance (RM-ANOVA), which analyzed the main effects of treatment and time and their interaction. Significant effects were further analyzed by Bonferroni corrected post-tests.

Results

Experiment 1: Acute Administration of a Drug or Drug Combinations to *Reduce* Repetitive Behavior. In the single drug experiments, we evaluated the efficacy of three doses of the dopamine D_2 receptor antagonist, L-471,626, two doses of the glutamate mGlu$_5$ receptor PAM, CDPPB, and one dose of the adenosine A_{2A} receptor agonist, CGS21680. In our primary analysis, repetitive behavior difference scores were not significantly different from vehicle in any of the single drug comparisons (Table 1). This nonsignificant effect of CGS21680 replicated our previous finding (Tanimura et al., 2010a). Our analyses of the double drug combinations revealed a similar outcome. No significant change in repetitive behavior difference scores, compared with vehicle, was found with the L-741,626 + CGS21680 combination, the L-741,626 + CDPPB combination, or the CGS21680 + CDPPB combination (Table 1).

A significant reduction in the repetitive behavior difference scores was found using the triple drug cocktail, L-741,626 + CGS21680 + CDPPB compared with vehicle ($t(11) = 3.99, P = 0.002$; Fig. 2). Our secondary analysis confirmed that the triple drug cocktail difference score was also significantly lower than the difference scores of all the other mice in the experiment that received vehicle ($t(112) = 3.80, P = 0.0002$). Furthermore, postinjection repetitive behavior counts (as opposed to difference scores) were also significantly lower following the triple drug cocktail, compared with vehicle, in that cohort ($t(11) = 3.87, P = 0.003$). This effect was selective for repetitive behavior and did not result from nonselective motor suppression, as revealed by our measure of total distance traveled in locomotor monitors in the hour following injection ($t(4) = 0.47, P = 0.66$).

Experiment 2: Acute Administration of a Drug or Drug Combinations to *Increase* Repetitive Behavior. Results from experiment 1 suggested that only a triple drug cocktail targeting the dopamine D_2, adenosine A_{2A}, and glutamate mGlu$_5$ receptor heteromers, which are located on the striatal indirect pathway neurons, could reduce repetitive behavior. As a proof of concept, we investigated whether a converse combination of drugs targeted to the heteromeric receptors could increase repetitive behavior. In the single drug experiments, we evaluated the dopamine D_2 receptor agonist, quinpirole, the adenosine A_{2A} receptor antagonist, SCH58261, and the glutamate mGlu$_5$ receptor NAM, MTEP. Repetitive behavior difference scores were not significantly altered by any of these single drug challenges compared with vehicle (Table 2). Furthermore, none of the double drug combinations were effective at increasing repetitive behavior difference scores either.

A triple drug cocktail of these drugs, quinpirole + SCH58261 + MTEP, resulted in significantly greater

TABLE 1

Difference scores for acute administration of drug or drug combinations to *reduce* repetitive behavior

Difference scores represent the number of repetitive behavior counts observed in the 60 minutes following injection minus the number of repetitive behavior counts observed in the 60 minutes preceding injection. Values are mean (S.E.M.).

| Cohort	n	Drug(s)	Dose(s) (mg/kg)	Vehicle Difference Score	Drug Difference Score	P Value
1-1	13	D_2 antagonist (L-741,626)	3	209 (275)	451 (259)	0.59
1-2	9	D_2 antagonist	10	789 (332)	231 (278)	0.37
1-3	13	D_2 antagonist	30	256 (235)	−83 (264)	0.30
1-4	11	mGlu$_5$ PAM (CDPPB)	3	94 (280)	−268 (204)	0.53
			30		−55 (283)	
1-5	12	A_{2A} agonist (CGS21680)	0.05	−199 (205)	−324 (226)	0.54
1-6	12	D_2 antagonist + A_{2A} agonist	30 + 0.05	44 (254)	−401 (382)	0.37
1-7	13	A_{2A} agonist + mGlu$_5$ PAM	0.05 + 30	451 (213)	−307 (297)	0.07
1-8	12	D_2 antagonist + mGlu$_5$ PAM	30 + 30	35(169)	−376 (187)	0.15
1-9	12	D_2 antagonist + A_{2A} agonist + mGlu$_5$ PAM	30 + 0.05 + 30	727 (237)	−703 (206)	0.002
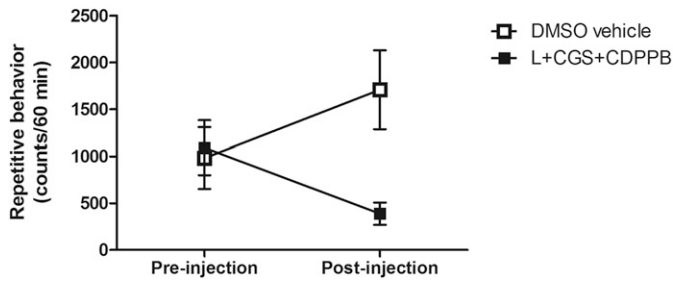
Fig. 2. Experiment 1: The triple drug cocktail made up of a D_2 antagonist (L-741,626 or L at 30 mg/kg), A_{2A} agonist (CGS21680 or CGS at 0.05 mg/kg), and mGlu$_5$ PAM (CDPPB at 30 mg/kg) reduced repetitive behavior counts for 1 hour postinjection. The same 12 deer mice received both the triple drug cocktail and 25% DMSO vehicle, separated by at least 1 week, in a random-order crossover design. Data are expressed as mean ± S.E.M.
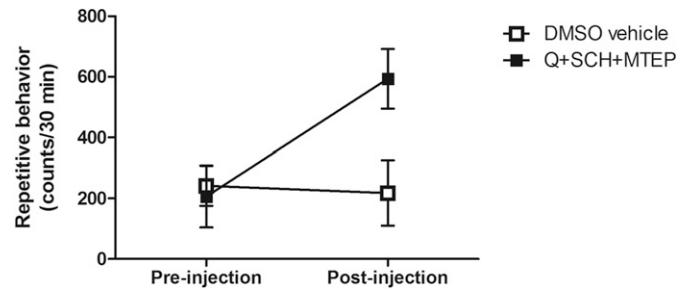

Fig. 3. Experiment 2: The triple drug cocktail made up of a D_2 agonist (quinpirole or Q at 3 mg/kg), A_{2A} antagonist (SCH58261 or SCH at 1 mg/kg), and mGlu$_5$ NAM (MTEP at 5 mg/kg) increased repetitive behavior counts for the 30 minutes following injection. The same seven deer mice received the triple drug cocktail and 10% DMSO vehicle, separated by at least 1 week, in a random-order crossover design. Data are expressed as mean ± S.E.M.

repetitive behavior difference scores than was observed with vehicle (t(6) = 2.45, $P < 0.05$; Fig. 3). Our secondary analysis confirmed that the difference scores of the triple drug cocktail mice were significantly higher than all of the other mice in the experiment when they received vehicle (t(42) = 4.20, $P = 0.0001$). Though when only considering postinjection repetitive behavior counts within the triple drug cocktail cohort (cohort 2-7), relative to their vehicle postinjection counts, there was only a trend toward significance (t(6) = 2.15, $P = 0.08$).

Experiment 3: Acute Administration of Depot Drug or Drug Combinations to *Reduce* Repetitive Behavior. Results from experiment 1 were encouraging but the duration of drug effect was relatively short, lasting only 1 hour. Drugs suspended in oleaginous solution have a longer duration of action, so we examined whether a peanut oil vehicle could extend the duration of action of the triple drug cocktail. Because hydrophilicity of the drugs changes their solubility in oil relative to aqueous solution, we retested each single drug dose to evaluate whether there were nonselective motor effects with the doses used in experiment 1. For L-741,626, the 30 mg/kg dose used in experiment 1 significantly reduced repetitive behavior counts in the 4-hours-postinjection assessment when suspended in peanut oil (t(12) = 5.82, $P < 0.0001$), and visual inspection of the video-recorded testing sessions revealed nonselective motor effects. Because of this, we evaluated decreasing doses of L-741,626 until we found a dose that did not cause obvious motor suppression. These doses of L-741,626 and their effect on repetitive behavior were: 15 mg/kg (t(10) = 4.33, $P = 0.002$), 7.5 mg/kg (t(8) = 2.87, $P = 0.02$), and 5 mg/kg (t(6) = 0.99, $P = 0.33$). We chose to use

the 5-mg/kg dose of L-741,626 for the double drug combination and triple drug cocktail tests because inspection of the video recorded testing sessions showed the mice were fully ambulatory. For CDPPB, the 30-mg/kg dose used in experiment 1 also significantly reduced repetitive behavior counts in the 4-hours-postinjection assessment when suspended in peanut oil (t(12) = 4.13, $P = 0.0014$), and like L-741,626, this effect on behavior was nonselective. Postinjection videos showed all overt motor behaviors were reduced. Following the dosing strategy of L-741,626, we then tested CDPPB at 15 mg/kg and found no significant effects on repetitive behavior counts. We chose to use the 15-mg/kg dose of CDPPB for the double drug combination and triple drug cocktail tests. For CGS21680, we found no significant effect of the 0.05-mg/kg dose used in experiment 1 when the drug was suspended in peanut oil. There was no evidence of any nonselective motor effects with the 0.05 mg/kg dose so we tested the efficacy of an increased dose, 0.3 mg/kg, of CGS21680. This dose also did not reduce repetitive behavior or show nonselective motor effects. We chose to use the 0.3-mg/kg dose of CGS21680 for the double drug combination and triple drug cocktail tests. In a subsequent evaluation of the double drug combinations, a crossover study showed no significant effect of any of the double drug combinations on repetitive behavior (Table 3).

Consistent with our finding in experiment 1, the triple drug cocktail significantly reduced repetitive behavior (t(10) = 3.17, $P = 0.01$; Table 3). By injecting the triple drug cocktail at the beginning of the dark cycle (i.e., 10:00 AM) we were able to determine that this significant reduction in repetitive behavior lasted 5 hours (Fig. 4). In addition, this effect was selective for repetitive behavior and was not owing to nonselective

TABLE 2

Difference scores for acute administration of drug or drug combinations to *increase* repetitive behavior

Difference scores represent the number of repetitive behavior counts observed in the 30 minutes following injection minus the number of repetitive behavior counts observed in the 30 minutes preceding injection. Values are mean (S.E.M.).

Cohort	n	Drug(s)	Dose(s) (mg/kg)	Vehicle Difference Score	Drug Difference Score	P Value
2-1	6	D_2 agonist (quinpirole)	3	−258 (230)	−207 (131)	0.79
2-2	6	mGlu$_5$ NAM (MTEP)	5	−26 (176)	−222 (160)	0.47
2-3	7	A_{2A} antagonist (SCH58261)	1	−86 (87)	−121 (139)	0.76
2-4	6	D_2 agonist + A_{2A} antagonist	3 + 1	−286 (106)	−781 (389)	0.17
2-5	6	A_{2A} antagonist + mGlu$_5$ NAM	1 + 5	−224 (92)	−14 (187)	0.15
2-6	6	D_2 agonist + mGlu$_5$ NAM	3 + 5	−85 (101)	85 (220)	0.60
2-7	7	D_2 agonist + A_{2A} antagonist + mGlu$_5$ NAM	3 + 1 + 5	−24 (173)	387 (48)	0.0497

motor suppression, as revealed by our measure of total distance traveled in locomotor monitors in the hour following injection ($t(5) = 0.22$, $P = 0.83$).

Experiment 4: Subchronic Administration of Depot Triple Drug Cocktail to *Reduce* Repetitive Behavior. To examine the long-term effectiveness of the triple drug cocktail in oil, we injected independent groups of deer mice each day for 7 days and measured their repetitive behavior on days 1, 4, and 7. A two-way RM-ANOVA revealed a significant main effect of drug ($F(1,36) = 14.57$, $P = 0.001$) and no significant main effect of time ($F(2,36) = 1.64$, $P = 0.21$) or drug × time interaction ($F(2,36) = 0.003$, $P = 0.99$). This shows that the triple drug cocktail continued to stay effective at reducing repetitive behavior over each of the test days and that rates of repetitive behavior did not change within either the drug or vehicle groups across time. The Bonferroni-corrected post-tests confirmed that the reduction in repetitive behavior by the triple drug cocktail was significant at each time point (Fig. 5).

There were no significant effects of the repeated triple drug cocktail injections on body weight ($F(1,108) = 0.04$, $P = 0.85$), though mice in both groups gained a small amount of weight across the 7 days of injections ($F(6,108) = 2.44$, $P = 0.03$) with no interaction ($F(6,108) = 0.78$, $P = 0.59$; Fig. 6A). For food intake, there was a significant main effect of drug ($F(1,36) = 44.19$, $P < 0.0001$) and significant main effect of time ($F(2,36) = 18.95$, $P < 0.0001$) but no significant drug × time interaction ($F(2,36) = 3.16$, $P = 0.05$). Both injection groups increased food intake across the three test days, and the triple drug cocktail group consistently consumed less food than the peanut oil vehicle group (Fig. 6B). This significant effect of the triple drug cocktail was also seen for water consumption ($F(1,36) = 29.87$, $P < 0.0001$; Fig. 6C), though there was no significant effect of time ($F(2,36) = 3.06$, $P = 0.59$) or a drug × time interaction ($F(2,36) = 0.99$, $P = 0.38$).

Discussion

Previous work using the deer mouse model has revealed hypofunction of the indirect basal ganglia pathway (Presti et al., 2003; Presti and Lewis, 2005; Tanimura et al., 2010a, 2011; Bechard et al., 2017). Studies using other models have also provided evidence for an important role for the indirect basal ganglia pathway in mediating repetitive behavior. For example, excessive grooming behavior expressed by the Shank3B mutant mouse was rescued by selective activation of indirect basal ganglia pathway neurons in the striatum (Wang et al., 2017). In addition, high frequency stimulation of the STN reduced excessive self-grooming in two mouse models relevant to autism spectrum disorder (Chang et al., 2016).
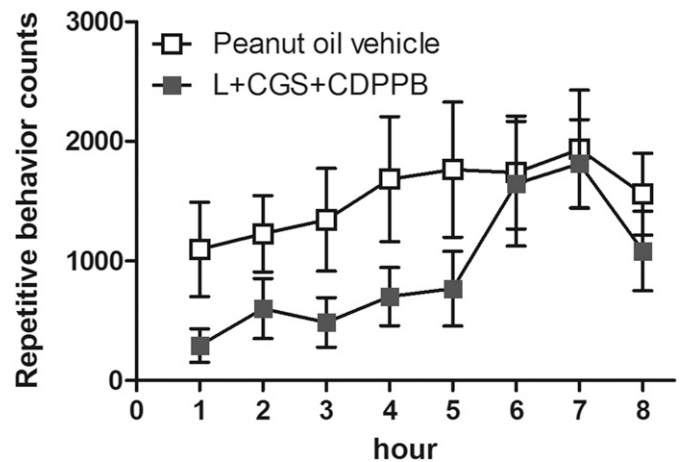

Fig. 4. Experiment 3: Time course of repetitive behavior counts following injection (given at $x = 0$, which represents 10:00 AM). The triple drug cocktail made up of a D_2 antagonist (L-741,626 or L at 5 mg/kg), A_{2A} agonist (CGS21680 or CGS at 0.3 mg/kg), and mGlu$_5$ PAM (CDPPB at 15 mg/kg) reduced repetitive behavior counts for 5 hours following injection. Ten deer mice received both the triple drug cocktail and the peanut oil vehicle in a random-order crossover design. Data are expressed as mean ± S.E.M.

Additionally, the STN of C58 mice, which exhibit the same topographies of repetitive behavior as deer mice, show many of the same pathologic characteristics of the deer mouse STN, namely reduced neuronal activation and dendritic spine density (Lewis et al., 2018).

We hypothesized that targeting dopamine D_2, adenosine A_{2A}, and glutamate mGlu$_5$ receptors found on striatopallidal neurons as a heteromeric complex (Cabello et al., 2009), would significantly alter repetitive behavior expression. In fact, we found that a triple drug cocktail designed to increase indirect basal ganglia pathway neuronal activation by preferentially targeting these receptors, significantly reduced repetitive behaviors, without affecting non-repetitive motor behaviors. Single drugs and double drug combinations were not effective, suggesting effects at all three receptors making up the heteromer were needed. The A_{2A} receptor agonist, CGS21680, had no significant effect on repetitive behavior either in saline or peanut oil. This contrasts with Amodeo et al. (2018) who found 0.01 mg/kg CGS21680 significantly reduced repetitive grooming behavior in BTBR mice. CGS21680 alone does not increase GABA release in the globus pallidus following intrastriatal injection (Beggiato et al., 2016), which suggests that CGS21680 alone does not increase striatal indirect basal ganglia pathway neuron neurotransmitter release.

TABLE 3

Repetitive behavior counts after acute administration of depot drug or drug combinations to *reduce* repetitive behavior

Values are 4 hours postinjection repetitive behavior count mean (S.E.M.). Doses of drugs that induced nonselective motor suppression are not shown.

| Cohort	n	Drug(s)	Dose(s) (mg/kg)	Vehicle	Drug	P Value
3-2	8	A_{2A} agonist (CGS21680)	0.05	5674 (1355)	4061 (621)	0.22
3-4	11	mGlu$_5$ PAM (CDPPB)	15	7453 (1255)	4651 (1083)	0.06
3-6	7	D2 antagonist (L-741,626)	5	7558 (1462)	6585 (1441)	0.37
3-7	11	A_{2A} agonist	0.3	6887 (1012)	6162 (1042)	0.18
3-8	10	D_2 antagonist + A2A agonist	5 + 0.3	4639 (1302)	3203 (1241)	0.08
		A_{2A} agonist + mGlu$_5$ PAM	0.3 + 15		3250 (1052)	
		D_2 antagonist + mGlu$_5$ PAM	5 + 15		3030 (1276)	
3-9	11	D_2 antagonist + A2A agonist + mGlu$_5$ PAM	5 + 0.3 + 15	5354 (1202)	2086 (719)	0.01
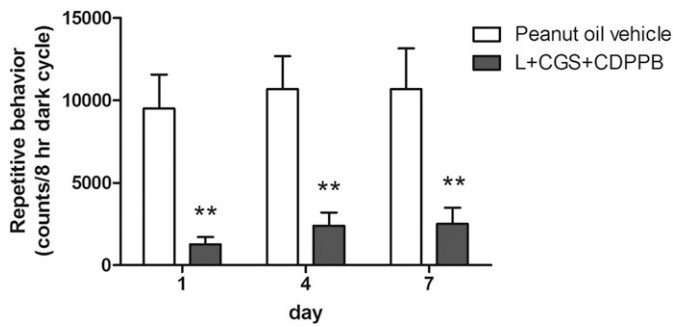
Fig. 5. Experiment 4: The triple drug cocktail made up of a D_2 antagonist (L-741,626 or L at 5 mg/kg), A_{2A} agonist (CGS21680 or CGS at 0.3 mg/kg), and mGlu$_5$ PAM (CDPPB at 15 mg/kg) was injected each day for 7 days and significantly reduced repetitive behavior counts when tested on days 1, 4, and 7, relative to the peanut oil vehicle group. Ten deer mice received triple drug cocktail and another 10 deer mice received peanut oil vehicle. RM-ANOVA was used and data are expressed as mean + S.E.M. (**$P <$ 0.01 for Bonferroni post-tests).

We also found that a converse drug cocktail strategy that consisted of a dopamine D_2 receptor agonist, an adenosine A_{2A} receptor antagonist, and a glutamate mGlu$_5$ NAM, significantly increased repetitive behavior. This demonstrates bidirectional effects on repetitive behavior expression through opposing actions on the indirect basal ganglia pathway. The effect of the converse drug cocktail is comparable to hemiparkinsonian rats that received A_{2A} and mGlu$_5$ antagonists during L-DOPA treatment, which significantly increased rates of rotational behavior (Fuzzati-Armentero et al., 2015). These data suggest that this converse drug cocktail increases motor output by reducing striatal indirect basal ganglia pathway function. Interestingly, mGlu$_5$ antagonists as single treatments have been shown to reduce repetitive self-grooming in BTBR mice, repetitive jumping in C58 mice (Silverman et al., 2010, 2012), and self-grooming and marble burying in the prenatal valproic acid model (Mehta et al., 2011).

One limitation of this study is that D_2, A_{2A}, and mGlu$_5$ receptors are expressed outside of the striatum and drug effects on these extra-striatal receptors may have played some role in the behavioral outcomes. Another limitation is that both the D_2 antagonist, L-741,626, and the D_2 agonist, quinpirole, used in these studies also bind to dopamine D_3 receptors, as these receptors share a high degree of sequence homology. Drug effects on dopamine D_3 receptors offer an interesting alternative to our hypothesized action of the triple drug cocktail on the D_2/A_{2A}/mGlu$_5$ receptor heteromer on the striatopallidal neurons in the dorsal striatum. Dopamine D_3 receptors also heteromerize with adenosine A_{2A} receptors (Torvinen et al., 2005), though predominantly in the ventral striatum where nearly all dopamine D_3 receptors are expressed (Sokoloff et al., 1990). Considerable evidence supports a preferential role for dorsal, rather than ventral, striatum in the expression of repetitive motor behavior. Given the present findings and the role of the indirect basal ganglia pathway involvement in repetitive behaviors, we suggest that the most likely drivers of the behavioral effects were the dopamine D_2 containing receptor heteromers. We must acknowledge, as well, that the significant effects on repetitive behavior could be due to changes in signaling of receptor monomers.

We also found that both short-acting (aqueous, DMSO vehicle solution) and long-acting (oleaginous, peanut oil vehicle solution) triple drug cocktail preparations were effective. In fact, administrating the triple drug cocktail in peanut oil extended the duration of action to 5 hours - an effect we were able to demonstrate by moving the time of injection to the beginning of the dark cycle. The peanut oil preparation also allowed us to reduce the doses of both L-741,626 and CDPPB in the double drug combinations and triple drug cocktail so that the potential concern of off-target effects of the drugs in experiment 1 could be attenuated, though not eliminated. Furthermore, our subchronic administration protocol continued to show reductions in repetitive behavior maintained across 7 days of injections, indicating that tolerance to the drugs does not occur with repeated administration. We also showed that repeated administration of the triple drug cocktail did not increase food or water consumption or weight gain, which is important because increased appetite and weight gain are significant side effects of the off-label antipsychotic drugs frequently prescribed to treat repetitive behaviors (McDougle et al., 1997; Potenza et al., 1999). In fact, the deer mice administered the triple drug cocktail had reduced food and water intake across all test days when compared with vehicle-treated deer mice. This decrease may have been the result of reduced energy expenditure related to lower repetitive behavior expression and should be followed up with proper measurements of energy intake and energy expenditure.
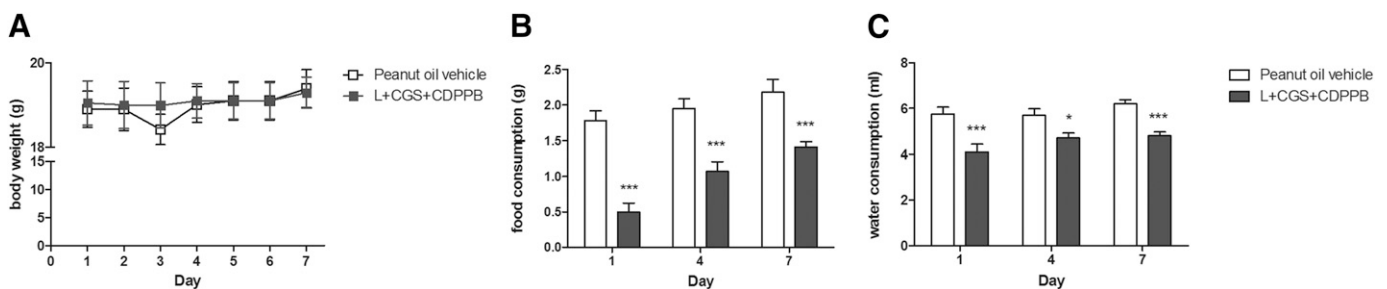

Fig. 6. Experiment 4: (A) The triple drug cocktail made up of a D_2 antagonist (L-741,626 or L at 5 mg/kg), A_{2A} agonist (CGS21680 or CGS at 0.3 mg/kg), and mGlu$_5$ PAM (CDPPB at 15 mg/kg) did not significantly change body weight across the 7 days of daily administration. (B) The triple drug cocktail group consumed less food than the peanut oil vehicle group on all testing days. (C) The triple drug cocktail group also consumed less water than the peanut oil vehicle group on all testing days. Ten deer mice received triple drug cocktail and another 10 deer mice received peanut oil vehicle. RM-ANOVA was used for each analysis and data are expressed as mean + or $\pm$ S.E.M. (*$P <$ 0.05; ***$P <$ 0.001, for Bonferroni post-tests).

Despite the high level of individual variability in repetitive behavior in deer mice, we have consistently failed to find a sex effect in repetitive behavior expression. The present studies used only male mice, which has the methodological advantage of limiting variability in both pharmacokinetic and pharmacodynamic response to drug. Nonetheless, this is a limitation of the present study and it will be important to demonstrate generalizability of the pharmacological effects obtained in female mice.

Our primary goal in conducting these experiments was to test further the role of the indirect basal ganglia pathway in mediating repetitive motor behavior. A second goal was to contribute to development of targeted pharmacotherapy for clinically relevant repetitive behaviors. Other candidates, on the basis of studies using single drugs with other animal models of repetitive behavior, include a 5-HT$_{2A}$ antagonist (Amodeo et al., 2016, 2017), 5-HT$_7$ and 5-HT$_{1A}$ partial agonists (Canal et al., 2015), a muscarinic acetylcholine agonist (Amodeo et al., 2014), and a GABA$_B$ agonist (Silverman et al., 2015). These single-drug studies notwithstanding, we think there are numerous advantages to a polypharmacy strategy targeted to heteromeric receptor complexes and multiple neurochemical systems (Cieslik et al., 2018; Podkowa et al., 2018). Targeting particular receptor complexes on specific neurons allows selectivity of activation and eliminates the necessity for high doses of single drugs that may bind to receptors in many regions of the brain and body (Rozenfeld and Devi, 2010). These subthreshold doses of different drug classes reduce the side effect profile of each drug and may improve the probability of safety and tolerability in neurodevelopmental, neurologic, and psychiatric populations who may exhibit drug sensitivity in a context of long-term use. This pharmacological strategy is also preferred over standard single target drugs because it takes advantage of the normal physiologic functioning of the cell. Numerous heteromeric complexes have been identified throughout the central nervous system and researchers are beginning to understand the differential functioning of receptor monomers and heteromers (Agnati et al., 2003; Brugarolas et al., 2014; Ferre et al., 2014; Gomes et al., 2016). Heteromeric receptor activation can have synergistic effects on cell signaling cascades such that targeting heteromeric receptor complexes has significantly more impact on cell functioning than single drug exposure (Popoli et al., 2001; Ferre et al., 2002).

Our finding of significant and selective reduction of repetitive behavior in deer mice with a triple drug cocktail designed to target a striatopallidal receptor heteromer is encouraging and suggests that further study of the mechanism of action is needed. We prepared the triple drug cocktail on the basis of our understanding of each receptor's association with cell signaling cascades and their effect on cellular activation (Ferre et al., 2002). Our future studies will evaluate which cell-signaling pathways and transcription factors mediate the positive pharmacotherapeutic effect. Adenosine A$_{2A}$ and glutamate mGlu$_5$ cascades use overlapping molecules, including mitogen-activated protein kinase and cAMP response element binding protein (Agnati et al., 2003). It will be important to understand which pathways are beneficial to drug response and may allow advancements in drug cocktail design that show promise in other fields like cancer drug development (Rashid et al., 2018). Pharmacokinetic and pharmacodynamic analyses of this triple drug cocktail are also imperative. We chose systemic administration for the translational value; however, intrastriatal

injections would help test the hypothesis that the indirect basal ganglia pathway neurons of the dorsal striatum are predominantly responsible for our behavioral effects. These future experiments will improve our understanding of the neurobiological mechanisms that mediate repetitive behavior reduction and will lead to the elucidation of more targets for novel pharmacotherapies.

Authorship Contributions

Participated in research design: Primiani, Lewis, Muehlmann.
Conducted experiments: Primiani, Muehlmann.
Contributed new reagents or analytic tools: Lewis.
Performed data analysis: Primiani, Muehlmann.
Wrote or contributed to the writing of the manuscript: Primiani, Lewis, Muehlmann.

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