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Indirect basal ganglia pathway mediation of repetitive behavior: Attenuation by adenosine receptor agonists

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Abstract

Repetitive behaviors are diagnostic for autism and common in related neurodevelopmental disorders. Despite their clinical importance, underlying mechanisms associated with the expression of these behaviors remain poorly understood. Our lab has previously shown that the rates of spontaneous stereotypy in deer mice (*Peromyscus maniculatus*) were negatively correlated with enkephalin content, a marker of striatopallidal but not striatonigral neurons. To investigate further the role of the indirect basal ganglia pathway, we examined neuronal activation of the subthalamic nucleus (STN) using cytochrome oxidase (CO) histochemistry in high and low stereotypy mice. CO activity in STN was significantly lower in high stereotypy mice and negatively correlated with the frequency of stereotypy. In addition, exposure to environmental enrichment, which attenuated stereotypy, normalized the activity of STN. Co-administration of the adenosine A_{2A} receptor agonist CGS21680 and the A_1 receptor agonist CPA attenuated stereotypy dose-dependently. The significant reduction associated with the lowest dose of the drug combination tested was due to its effects on mice with lower baseline levels of stereotypy seen. Higher doses of the drug combination were required to show robust behavioral effects, and presumably requisite activation of the indirect pathway, in high-stereotypy mice. These findings support that decreased indirect pathway activity is linked to the expression of high levels of stereotypy in deer mice and that striatal A_1 and A_{2A} receptors may provide promising therapeutic targets for the treatment of repetitive behaviors in neurodevelopmental disorders.

Six key words

autism; stereotypy; subthalamic nucleus; neurodevelopmental disorders; deer mice; animal models

Introduction

Restricted, repetitive behaviors are typically characterized as inflexible, persistent, and apparently functionless. This categorization captures a wide range of behaviors from sensorimotor (e.g., motor stereotypy, repetitive manipulation of objects, compulsions) to more cognitively driven behaviors (e.g., insistence on sameness, restricted interests) [10,51]. Restricted, repetitive behaviors constitute one of three diagnostic domains of autism spectrum disorder ([30] for review) and are common features of related neurodevelopmental disorders (e.g., Rett's syndrome, intellectual and developmental disability) as well as several psychiatric

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disorders (e.g., obsessive-compulsive disorder (OCD), trichotillomania) and neurological diseases (e.g., Tourette syndrome, frontotemporal dementia). In addition, motor stereotypies have been described in adults and children without diagnosed neurodevelopmental, psychiatric, or neurological disorders [4,5,32,48] and are ubiquitous in normative development [12,54].

Despite their clinical importance, the specific neurobiological mechanisms associated with the abnormal expression of these behaviors are largely unidentified. Several neuroimaging studies have identified structural and functional basal ganglia differences linked to the expression of repetitive behaviors [21,33,35,46]. Experimental manipulations known to induce stereotypies in animals have provided more direct evidence for the importance of cortico-basal ganglia circuitry in mediating these behaviors (e.g., [57]).

Dysregulation of cortico-striato-thalamo-cortical circuitry associated with motor disorders is thought to be due to an imbalance between the direct and indirect pathways comprising this circuitry [17]. For example, injection of a GABA antagonist to the external aspect of the globus pallidus (GPe) induced stereotypy in non-human primates, which was attenuated by deep brain stimulation (DBS) of the subthalamic nucleus (STN) [3]. Similarly inactivation of STN exacerbated the compulsive lever-pressing observed in rats in the signal attenuation model of OCD [59]. These results point to a potentially important role for the indirect pathway function in the expression of repetitive behavior of unknown etiology.

Our laboratory has employed deer mice (*Peromyscus maniculatus*) which develop high levels of motor stereotypies (repetitive jumping and/or backward somersaulting) as a consequence of being reared in a standard laboratory environment. These behaviors, which do not require social isolation, specific cues or contexts, pharmacological agents, or specific CNS insult for induction, occur at a high rate, persist across much of the life of the animal and appear relatively early in development. Housing these mice in more complex environments especially early in development attenuates the development and expression of these behaviors [18,38]. The repetitive hindlimb jumping and backward somersaulting exhibited by deer mice correspond to the repetitive sensorimotor behaviors frequently observed in neurodevelopmental disorders. We have also shown that highly stereotypic deer mice have deficits in a reversal learning task, mirroring inflexibility and resistance to change [53]. These features plus considerable heterogeneity in individual levels of expression, modulation by early experience, and mediation by cortical-basal ganglia circuitry make deer mice a useful model of restricted, repetitive behavior in neurodevelopmental disorders.

We have previously shown that alterations of cortico-basal ganglia circuitry are linked to the expression of spontaneous repetitive behaviors in deer mice [39,41,55]. In particular, we found a significant inverse correlation between striatal enkephalin content and stereotypy. Conversely, there was no association between dynorphin and repetitive behavior [40]. This finding lead to the hypothesis that the expression of spontaneous stereotypy is a result of imbalanced activity of striatal pathways driven by reduced activity of the indirect pathway.

In the present study, we conducted two sets of experiments to assess the function of the indirect pathway in the expression of stereotypy. First, we assessed the neuronal metabolic capacity of STN as an index of indirect pathway activity using cytochrome oxidase (CO) histochemistry in adult deer mice (Experiment 1a). We repeated the same analysis in animals which were either housed under standard laboratory or environmentally enriched conditions (Experiment 1b). CO activity reflects oxidative metabolic capacity of neurons and has been shown to be directly related to neuronal functional activity and has been used to identify neuronal pathways activated by experience [44]. Moreover, unlike 2-DG or c-Fos, CO histochemistry reflects sustained alterations in neuronal activity. In addition, Hevner and Wong-Riley [20] have shown

that the optical density of histochemically labeled brain sections closely correlates with the amount of CO in CNS tissue.

Second, we pharmacologically manipulated striatopallidal neuronal activity via adenosine A_{2A} receptors which are highly enriched in the striatum and selectively expressed in these neurons [13,22,45]. Karcz-Kubicha et al. [23,24] reported that administration of an A_{2A} agonist alone failed to induce c-Fos in the striatum, whereas co-administration of the same A_{2A} agonist plus an A_1 agonist resulted in significant increase in striatal c-Fos expression as well as enkephalin expression. Importantly, this increased expression was observed in striatopallidal but not striatonigral neurons. Thus, we assessed the effect of an adenosine A_{2A} agonist alone (Experiment 2a) as well as the combination of an A_{2A} receptor agonist plus A_1 receptor agonist (Experiment 2b, c, and d) on the attenuation of the expression of stereotypy.

General methods

Subjects

All deer mice (*Peromyscus maniculatus*) were obtained from the breeding colony maintained in our laboratory, and kept on a 16:8-h light/dark cycle with lights off at 10:00 AM. Mice were weaned at 21 days of age. Rodent chow and water were available *ad libitum*. The room was maintained at 20–25°C and 50–70% humidity. When housed in standard laboratory cages, deer mice exhibit stereotyped behaviors in the form of hindlimb vertical jumping or backward somersaulting with considerable between animal variability. All procedures were performed in accordance with the guidelines set forth in the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Florida Institutional Animal Care and Use Committee.

Stereotypy Assessment

Rates of spontaneous stereotypy were assessed using a modified automated photocell detection apparatus (Columbus Instruments). The session consisted of the eight hours of the dark cycle. Mice were individually placed in testing cages (22 × 28 × 25 cm) made of Plexiglas and habituated for at least one hour prior to the beginning of the dark cycle. Food and water were provided. Stereotypy counts, which equated to the number of vertical jumps or backward somersaults a deer mouse performed during that 8 hours, were automatically scored for each mouse. Photobeams were positioned (13.5cm above the floor) to be interrupted by the vertical motion of jumping and somersaulting but not by rearing. All sessions were digitally video-recorded for identification of behavioral topographies and accuracy of the automated counters. Each animal received a stereotypy score that represented the average stereotypy frequency per hour.

Data Analysis

Differences in CO activity between high- and low-stereotypy mice and between enriched and standard caged mice were assessed by t-test with CO differences in STN being the primary comparison of interest. The association between the frequency of spontaneous stereotypies and the CO activity were analyzed by Pearson correlation (Experiment 1). Behavioral responses to pharmacological manipulations were analyzed by ANOVA and t-test (Experiment 2). All tests were two-tailed and effects were considered significant when $p<0.05$.

Specific Methods and Results

Experiment 1

Experiment 1a: Neuronal Activation of STN in High- and Low-Stereotypy Mice

Methods: Twenty-one adult mice (>8 weeks post-weaning; 15 males and 6 females) exhibiting repetitive vertical jumping were used. All mice were group-caged (5–6 mice per cage) from weaning in standard rodent cages (48 × 27 × 15 cm). Each mouse was assessed for stereotypy as described previously and separated into two groups (high and low stereotypy) based on a median split of stereotypy scores.

Following behavioral assessment, mice were killed and their brains were quickly removed and frozen by immersing them in cold 2-methylbutane. They were stored at −80°C until cryostat sectioning. The sections were cut sagittally at 20μm at −20°C starting approximately 2.7 mm lateral to the midline and collected every 100μm for both hemispheres. Sections were mounted on pretreated slides (Superfrost plus, Fisher), and stored in the −80°C freezer until assayed.

The CO spectrophotometric analysis and staining assay was carried out according to the Gonzalez-Lima protocol [15]. Standards were cut at five thicknesses (20, 30, 40, 50, and 60μm) and assayed with other brain sections. The CO staining was quantified by densitometric analysis using ImagePro (Media Cybernetics), and standards were used to convert optical density values into enzymatic activity values (μmol/min/g). Multiple optical density readings were made per animal for STN as well as for other areas comprising cortico-basal ganglia circuitry (motor cortex, striatum, substantia nigra pars reticulata (SNpr), substantia nigra pars compacta (SNpc)) and negative controls (hippocampus, somatosensory cortex). Dorsal and ventral aspects of the striatum were defined by horizontal bisection into approximately equivalent halves, whereas medial versus lateral aspects were defined by approximately <2.0mm or >2.0mm from the midline respectively. Under our experimental conditions, the staining intensity was highly correlated to the thickness of the standard sections (r =0.95).

Results: Baseline stereotypy scores varied from 137.9 to 3992.8 counts per hour with a median score of 652.0. The average score was 394.1 for low-stereotypy animals (n=11) and 1393.0 for high-stereotypy animals (n=10).

CO enzymatic activity within selected areas is summarized in Table 1. Significant differences were found in STN ($t(19)=2.74$, $p=0.01$) where low-stereotypy mice showed higher CO activity compared to high-stereotypy mice. Similar differences were found in the ventromedial striatum ($t(19)=2.21$, $p=0.04$) and SNpr ($t(19)=2.25$, $p=0.04$). In addition, individual levels of stereotypy were negatively correlated with CO activities in STN ($r=-0.50$, $p=0.02$) (Fig 1) and SNpr ($r=-0.53$, $p=0.01$). When the highest stereotypy score was excluded based on its value being more than 2 SDs greater than the mean, the correlations with CO were no longer significant (STN: $r=-0.41$, $p=0.07$; SNpr: $r=-0.41$, $p=0.07$). In addition, without this outlying score, the differences in the ventromedial striatum and SNpr between high and low stereotypy mice were no longer statistically significant ($t(18)=1.96$, $p=0.07$; $t(18)=1.91$, $p=0.07$ respectively).

Experiment 1b: Effects of Enriched Environment on Stereotypy and Neuronal Activation in STN

Methods: Twenty-three mice (14 males and 9 females) were group-caged (5–6 mice per cage) in standard rodent cages (48 × 27 × 15 cm) at weaning for 30 days, and then tested for baseline levels of stereotypy as described in the previous section. After testing, animals were randomly assigned to one of two housing conditions: standard rodent cages (SC) (n=12) or environmental enrichment (EE) (n=11). The EE housing consisted of large dog kennels (122 × 81 × 89 cm) with two extra levels of floors constructed of galvanized wire mesh and connected by ramps

of the same material. Bedding, a running wheel, shelters, and various other objects were placed in each kennel. In addition to *ad libitum* food and water, one oz. of Cockatiel vita seed was scattered throughout the kennel three times each week to encourage foraging behavior. A running wheel remained undisturbed in the kennel, but other objects were removed and replaced with clean novel objects on a weekly basis. The SC housing took place in the same rodent cages described above except that each cage contained 2–3 mice. They received *ad libitum* food and water as well as Cockatiel vita seed placed at one corner on the same schedule as the EE housing.

Animals were kept in their respective housing conditions for 30 days, during which time handling was kept to a minimum. Each mouse was tested again for stereotypy at the end of the EE or SC housing. Their brains were collected for the CO assessment as described previously.

Results: Mean baseline stereotypy scores assessed 30 days post-weaning were virtually identical for the animals assigned to SC versus EE ($t(21)=0.05$, $p=0.96$; see Fig 2). The subsequent 30 days of environmental enrichment significantly attenuated the development of stereotypy, whereas rates of stereotypy continued to increase in SC animals ($t(21)=-3.68$, $p<0.01$).

CO enzymatic activity within selected brain regions is summarized in Table 2. Significant differences were found in STN ($t(21)=3.57$, $p<0.01$) as well as SNpc ($t(21)=4.16$, $p<0.01$) and SNpr ($t(21)=3.74$, $p<0.01$). In these nuclei, EE mice had higher CO activities compared to SC mice.

Experiment 2

Experiment 2a: Effects of CGS21680 on Stereotyped Behavior

Methods: Thirty-nine male mice (> 6 weeks post weaning) were randomly assigned to one of four groups and were administered an acute subcutaneous injection of vehicle (n=13), 0.02mg/kg (n=9), 0.03mg/kg (n=9), or 0.05mg/kg (n=8) of 2-p-(2-carboxyethyl)phenethylamino-50-N-ethylcarboxamidoadenosine (CGS21680) (Sigma) dissolved in 5% dimethylsulfoxide (DSMO) in a volume of 10ml/kg of body weight. Drug injections were given two hours before the end of the dark cycle, a time period during which deer mice show higher rates of spontaneous stereotypy (unpublished data). The behavioral response to the drug was assessed starting immediately after drug administration.

Results: Administration of CGS21680 did not significantly alter the frequency of stereotyped behaviors for the 1hr post-injection period at doses up to 0.05mg/kg ($F(3,35)=1.45$, $p=0.25$). A preliminary study with a small number of animals indicated that CGS21680 at the 0.1mg/kg level had a non-selective effect on stereotypy as this dose suppressed motor activity in general. Similar effects were also seen during the 30min post-injection period.

Experiment 2b: Effects of Co-administration of CGS21680 and CPA on Stereotyped Behavior

Methods: Eighteen male mice (6 to 8 weeks post-weaning) were administered acute subcutaneous injections of a combination of CGS21680 and the selective A_1 receptor agonist N6-cyclopentyladenosine (CPA) (Sigma) dissolved in 5% DSMO. Each mouse received each drug combination (0.03/0.03; 0.05/0.05; and 0.1/0.1mg/kg) and vehicle only, serving as its own control. Each injection was administered one-week apart using successively higher doses and randomizing the order of drug and vehicle. For Experiment 2 (b, c, and d), we employed new testing cages (22 × 28 × 31 cm) made of Plexiglas to replace the old testing cages (22 × 28 × 25 cm).

Results: For all the drug combinations tested (n=18), there was no difference in stereotypy between vehicle and drug groups for the 1hr pre-injection period at 0.03/0.03mg/kg ($t(17)=3.28$, $p<0.01$), 0.05/0.05mg/kg ($t(17)=-0.60$, $p=0.56$), and 0.1/0.1mg/kg ($t(17)=-0.32$, $p=0.75$). For the 1hr post-injection period, drug groups showed significantly lower rates of stereotypy at 0.03/0.03mg/kg ($t(17)=3.97$, $p<0.01$), 0.05/0.05mg/kg ($t(17)=5.48$, $p<0.001$), and 0.1/0.1mg/kg ($t(17)=10.09$, $p<0.001$) when compared to their respective vehicle conditions (Fig 3). Similar drug effects were also seen during the 30min post-injection period.

To characterize this result further, we used the total pre-injection stereotypy score to categorize mice into high- and low-stereotypy groups based on a median split (n=9 each), and we assessed whether rates of baseline stereotypy predicted the magnitude of the drug response. At the 30min period, there was no difference from pre-injection baseline in high-stereotypy mice ($t(8)=0.97$, $p=0.36$) whereas the reduction from baseline for low-stereotypy mice approached significance ($t(8)=2.10$, $p=0.07$). This was also seen for the 1hr period in high-stereotypy mice ($t(8)=1.00$, $p=0.35$) and in low-stereotypy mice ($t(8)=1.91$, $p=0.09$).

The percentage change from baseline also showed that 0.03/0.03mg/kg of CGS21680/CPA was more effective in low-stereotypy mice (63.9% versus 3.5% for vehicle) than in high-stereotypy mice (12.4% reduction versus 0.6% for vehicle). No differential response was seen in high versus low baseline stereotypy mice at 0.05/0.05mg/kg and 0.1/0.1mg/kg.

Experiment 2c: Effects of CGS21680 and CPA in Drug-Naïve Animals

Methods: An independent group of 10 male mice (6 to 8 weeks post-weaning) was used to provide a partial replication of the results described in the prior experiment. In this case, we assessed the effects of 0.05/0.05mg/kg versus vehicle in drug-naïve animals using a cross-over design.

Results: Consistent with what we found previously, there was no difference in the frequency of stereotypy between the drug and vehicle conditions for the 1hr pre-injection periods ($t(9)=0.49$, $p=0.64$). Drug-treated animals, however, exhibited significantly less stereotypy for the 1hr post-injection periods compared to vehicle ($t(9)=3.40$, $p=0.01$ respectively) (Fig 4). Thus the effect of CGS21680 and CPA within dose combination was replicated in drug-naïve mice. The similar drug effects were also seen during the 30min post-injection period.

To determine that the effects of CGS21680/CPA were not solely due to CPA alone, we tested the effect of CPA at 0.05mg/kg using an independent group of animals (n=12) in a crossover design. Administration of CPA alone at 0.05mg/kg induced non-selective motor supression, rendering about a third of animals akinetic.

Experiment 2d: Effects of CGS21680 and CPA on Locomotor Behavior

Methods: To assess how selective the effect of the drug combination was on stereotyped behaviors, the animals from Experiment 2c received an additional administration of vehicle and the CGS21680 and CPA combination (0.05/0.05 and 0.1/0.1mg/kg) in a cross-over design. Their post-injection locomotor activity was tracked for 1 hr using Ethovision (Noldus). One control animal for the 0.05/0.05mg/kg group was excluded from the analysis due to missing data (n=9).

Results: There was a significant difference in distance traveled (cm) for the duration of the 30min post-injection period ($t(8)=2.70$, $p=0.03$) but not for the 1hr post-injection period ($t(8)=1.88$, $p=0.10$) between vehicle and 0.05/0.05mg/kg groups (Table 3A). There was significant reduction in distance traveled for the 30min post-injection period ($t(9)= 4.85$, $p=0.001$) and the 1hr post-injection period between vehicle and 0.1/0.1mg/kg groups ($t(9)= 4.58$, $p=0.001$)

(Table 3B). There was no difference between 0.05/0.05mg/kg and 0.1/0.1mg/kg groups, however, at either time point. Locomotor activity was significantly correlated with the frequency of stereotypy for the 1hr post-injection period in vehicle-treated mice (r=0.89, p<0.001) but not in drug-treated mice (r=0.51, p=0.13) at 0.05/0.05mg/kg.

Discussion

The present experiments were designed to test the hypothesis that the expression of spontaneous stereotypy in deer mice is linked to decreased activity of the indirect basal ganglia pathway. This hypothesis was based on our previous finding that level of stereotypy in deer mice was negatively correlated with the striatal expression of the neuropeptide enkephalin, whereas no relationship was found between stereotypy and the expression of dynorphin [40].

In support of this hypothesis, CO activity in STN was significantly lower in high-stereotypy mice compared to low-stereotypy mice (Experiment 1a) and in SC mice compared to EE mice (Experiment 1b).

Contrary to our expectation, no difference in CO activity between high- and low-stereotypy mice was found in the dorsolateral striatum, which is typically considered the sensorimotor area of the striatum. The medial aspect of the dorsal striatum, however, showed lower CO activity in high- compared to low-stereotypy mice (Experiment 1a), although a similar difference in the dorsomedial striatum was not found between SC and EE mice (Experiment 1b). The dorsomedial striatum has been implicated in the mediation of behavioral flexibility [43]. We have previously shown that high rates of stereotyped jumping in deer mice were associated with perseverative behavior in a reversal learning task [53], further supporting alterations of the dorsomedial striatum in stereotypic deer mice.

In addition, we also found significantly higher CO activity in SNpc and SNpr in EE versus SC mice (Experiment 1b). For SNpc, this effect is likely due to the monosynaptic excitatory STN-SNpc projection in rodents and non-human primates widely reported in the literature [8,19, 26,47]. This difference, however, was not found between high- and low-stereotypy mice (Experiment 1a). For SNpr, increased CO staining was associated with lower stereotypy in both Experiments 1a and 1b. These differences are consistent with higher glutamatergic activation from STN.

If high rates of spontaneous stereotypy are associated with decreases in indirect pathway activation, then stimulation of A_{2A} receptors, which are expressed on striatopallidal neurons and activate Gs/olf proteins upon stimulation, should attenuate stereotypy. When administered alone, the selective A_{2A} receptor agonist CGS21680 failed to reduce stereotypy up to 0.05mg/kg (Experiment 2a). In a preliminary study, 0.1mg/kg of CGS21680 non-selectively reduced motor activity, rendering some mice akinetic. The addition of CPA to CGS21680, however, selectively attenuated stereotypy in a dose-dependent manner without adverse suppression of general motor activity (Experiment 2b, c, and d) even at the 0.1 mg/kg dose. Although the lowest dose of CGS21680/CPA significantly attenuated stereotypy compared to vehicle controls, a subsequent analysis suggested that it was not effective in mice exhibiting higher rates of baseline stereotypy. Higher doses of CGS21680/CPA were required to attenuate stereotypy in this group. These pharmacological results indicate that higher doses of the drug combination may be required to drive the activity of the indirect pathway to levels associated with low stereotypy.

The relative efficacy of the combined stimulation of A_{2A} and A_1 receptors compared to A_{2A} alone may be explained by the results reported by Karcz-Kubicha et al. [23,24]. In this work, administration of an A_1 or A_{2A} receptor agonist alone did not induce striatal c-Fos expression. Stimulation of both receptor subtypes, however, did induce striatal c-Fos expression and in a

selective fashion with activation seen in striatopallidal, but not striatonigral, neurons. This combined treatment of A_1 and A_{2A} receptor agonists also increased striatal enkephalin expression. It should be noted, however, that these effects were seen at higher doses (0.5 mg/kg CGS21680 and 0.3 mg/kg CPA) that were used in the present experiments. Similarly, administration of an A_{2A} receptor antagonist alone produced either no effect or only a slight decrease in c-Fos and *preproenkepahlin* expression in the striatum [1,2,24,29,31,36,50,56]. This is consistent with our unpublished data showing that administration of the A_{2A} receptor antagonist SCH58261 failed to induce or exacerbate repetitive behavior.

The mechanisms to account for these effects likely involve functional antagonistic interactions of A_{2A} and D_2 receptors on adenynyl cyclase that regulate the cAMP-PKA signaling pathway in striatopallidal neurons. Tonic inhibition of D_2 receptors attenuates the ability of an A_{2A} agonist to stimulate this signaling pathway and its downstream effects on c-Fos and *preproenkephalin*. Moreover, A_{2A} receptor agonist administration has been reported to increase striatal dopamine release, possibly indirectly through A_{2A} receptors on presynaptic glutamate terminals. [14,24,42]. Conversely, A_1 receptor agonist administration has been reported to decrease striatal dopamine release [34,42] through A_1 receptors on presynaptic dopaminergic terminals where upon stimulation, release of dopamine is directly inhibited via activation of Gi/o protein [6,24,61]. Stimulation of presynaptic A_1 receptors by CPA allows A_{2A} receptors on striatopallidal neurons to overcome tonic inhibition by D_2 receptors to activate the cAMP-PKA pathway. Moreover, the effect of the A_1 and A_{2A} receptor heteromeric complex on glutamate release from corticostriatal neurons is dependent on the local concentration of adenosine [9], and so the drug effects reported here may also be due, at least in part, to alterations in glutamate release.

The biochemical and pharmacological findings presented here provide additional, important support for the role of the indirect pathway in mediating repetitive behavior in our model. These findings are consistent with both clinical and animal studies that have found a link between repetitive behavior and the indirect basal ganglia pathway. For example, the uncontrolled motor movements characteristic of Huntington's disease are attributed to the differential degeneration of striatopallidal neurons [11,49]. Deep brain stimulation (DBS) applied to STN reduced the severity of symptoms in previously treatment refractory OCD patients [7]. Similarly, DBS of STN and GPe has been found to be effective in ameliorating the L-DOPA induced tardive dyskinesia observed in Parkinson's disease (e.g., [28]). In animal models, Grabli et al. [16] have reported that stereotyped behavior (e.g., licking and biting of fingers) was induced in monkeys when the GABA antagonist bicuculline was microinjected into the limbic aspect of the GPe, which was reduced by DBS applied to STN without affecting a control motor task [3]. Winter et al. [59] have shown that rats that sustained ibotenic acid lesions to STN exhibited an increase in compulsive lever pressing in the signal attenuation model of OCD. This same research group has also shown that bilateral high frequency stimulation of STN as well as pharmacological inactivation of STN reduced quinpirole-induced compulsive checking in rats [27,58,60].

In addition to identifying pathophysiological changes associated with repetitive behavior, our pharmacological findings also point to novel targets for development of drug therapies to treat repetitive behavior in clinical disorders such as autism. Currently, the two drug classes used to treat such behaviors in neurodevelopmental disorders include atypical antipsychotics and selective serotonin re-uptake inhibitors (SSRIs). A recent multi-site study of the SSRI citalopram provided no evidence for its efficacy in treating repetitive behavior in autism [25]. There is limited evidence for the utility of atypical antipsychotics in treating repetitive behavior. Risperidone has recently been FDA approved for use in autism to treat irritability with no approval sought for repetitive behavior. Thus, there is a pressing need for the development of

effective drug treatments, based on an understanding of the specific pathophysiological mechanisms of repetitive behaviors.

The present finding suggests that a combination of A_{2A} receptor agonist and A_1 receptor agonist to drive indirect pathway activity reduce repetitive behaviors selectively, whereas either drug alone induce no or non-selective effect on behaviors respectively. Although cardiovascular relaxation induced by adenosine receptor agonists could be a possible drawback (e.g., [37, 52]), these findings suggest drug development efforts that may be potentially useful in treating repetitive behaviors in neurodevelopmental disorders.

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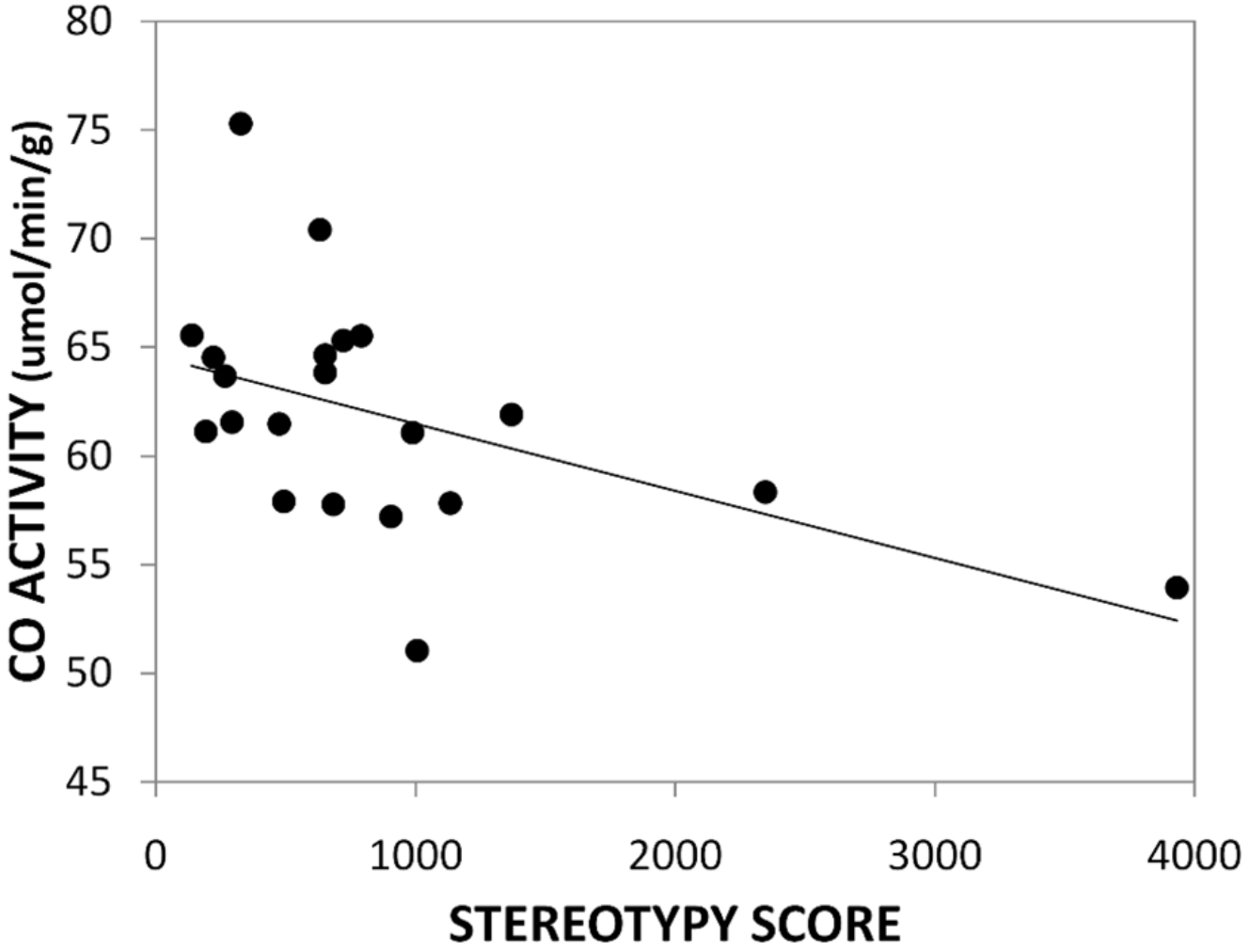

Fig 1.
Correlation between the frequency of stereotypy and CO activity in STN.

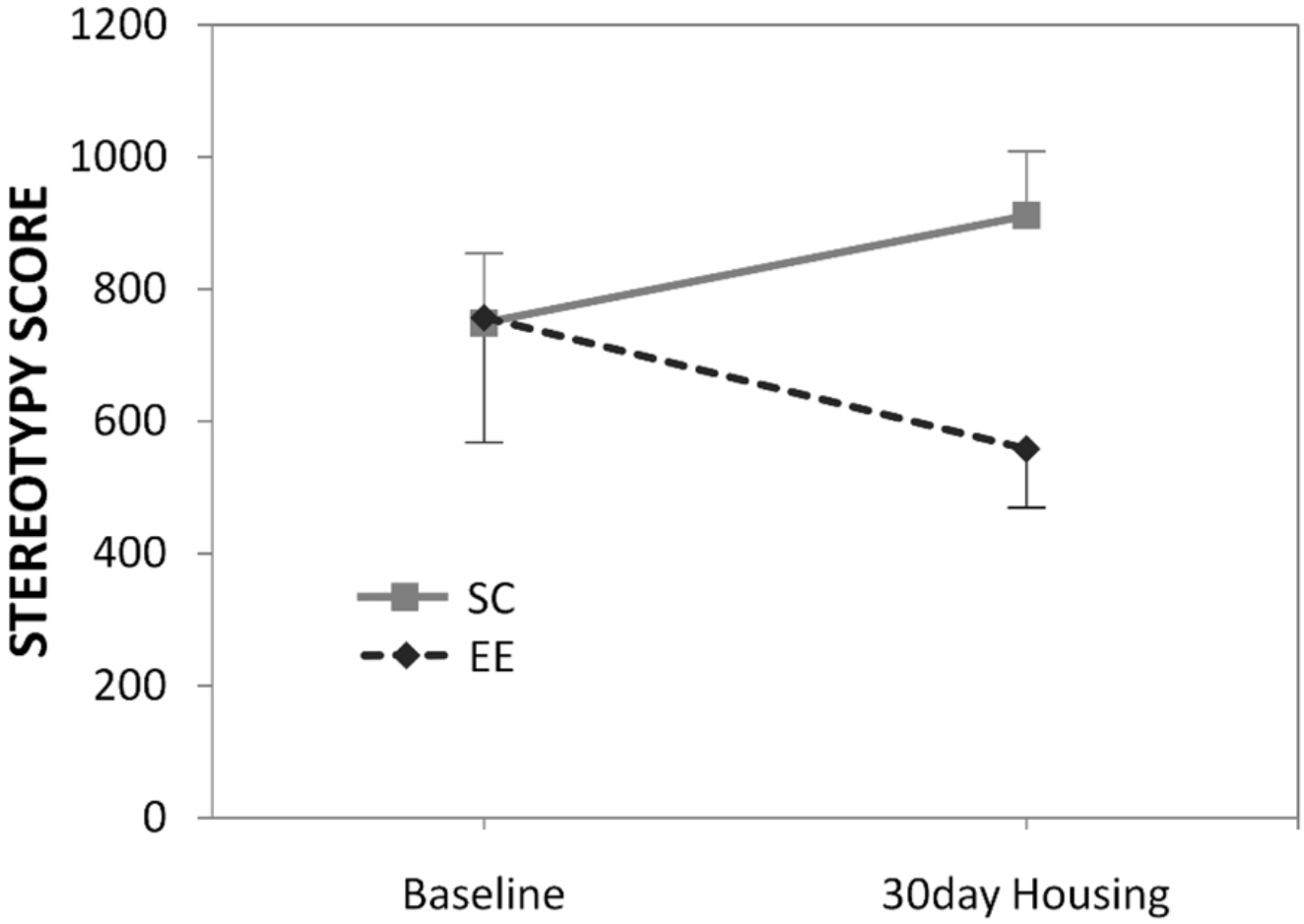

Fig 2.
The effect of 30-day EE housing on the stereotypy scores (mean ± SEM).

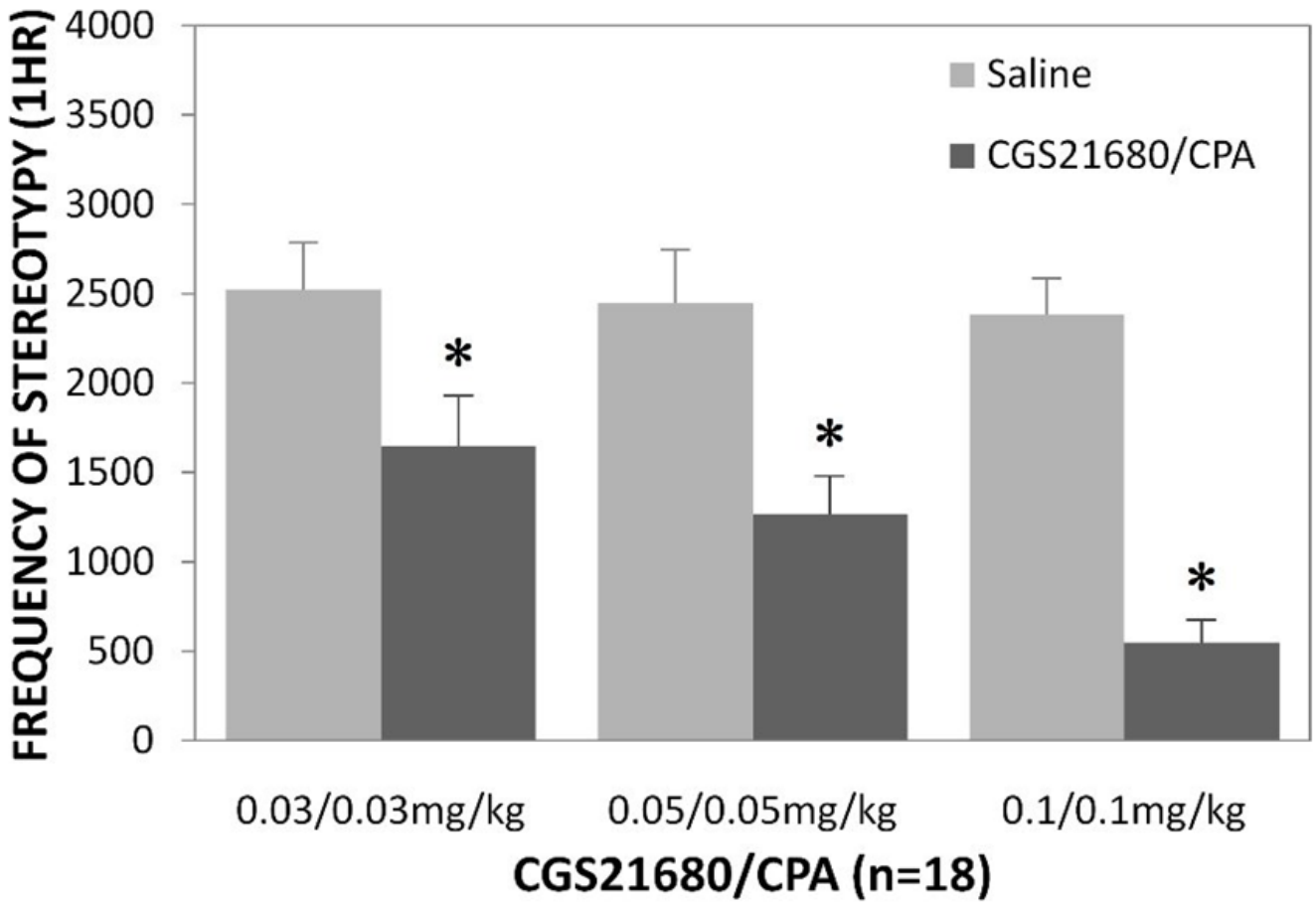

Fig 3.

The effects of CGS21680/CPA on stereotypy. The frequency of stereotypy for the 1hr post-injection period (mean ± SEM). * represents statistical significance at $p<0.05$ as compared to saline group.

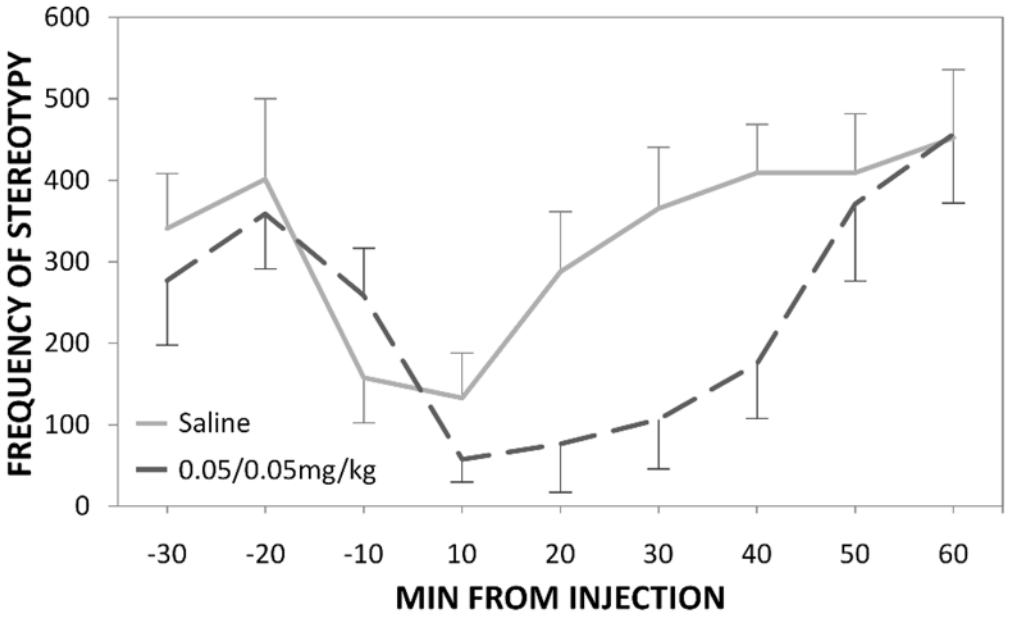

Fig 4.

The time-course showing the efficacy of CGS21680/CPA in drug-naïve animals (0.05/0.05mg/kg). Zero at the time of drug or saline injection (mean ± SEM). * represents statistical significance at $p<0.05$ as compared to saline group.

Table 1

CO activity (μmol/min/g) of STN and the other brain areas in high- and low-stereotypy adult deer mice. Values expressed are group means with SEM in parentheses.

	Low Stereotypy	High Stereotypy	*P*-value
Subthalamic Nucleus	**64.60 (1.42)**	**59.01 (1.46)**	**0.01**
Motor Cortex	48.01 (0.88)	47.84 (1.12)	0.89
Somatosensory Cortex	49.64 (0.74)	49.67 (1.25)	0.98
Hippocampus	44.12 (1.12)	42.46 (1.79)	0.80
Caudate/Putamen			
Dorsolateral Striatum	48.48 (0.81)	47.87 (1.42)	0.71
Dorsomedial Striatum	49.30 (1.02)	46.76 (1.12)	0.10
Ventrolateral Striatum	49.43 (0.74)	48.25 (1.08)	0.37
Ventromedial Striatum	50.28 (1.15)	46.96 (0.98)	**0.04**
Nucleus Accumbens	50.85 (1.12)	48.08 (1.02)	0.09
Substantia Nigra pars Compacta	45.10 (1.66)	42.22 (0.91)	0.16
Substantia Nigra pars Reticulata	50.72 (1.66)	46.25 (1.05)	**0.04**

Table 2

CO activity (μmol/min/g) of STN and the other brain areas in deer mice housed in the SC and EE conditions. Values expressed are group means with SEM in parentheses.

	SC	EE	*P*-value
Subthalamic Nucleus	**64.23 (0.71)**	**68.97 (1.15)**	**<0.01**
Motor Cortex	51.36 (0.58)	50.62 (0.64)	0.40
Somatosensory Cortex	51.36 (0.68)	51.19 (0.68)	0.86
Hippocampus	42.73 (1.12)	44.83 (0.81)	0.15
Caudate/Putamen			
Dorsolateral Striatum	52.31 (0.91)	52.92 (0.85)	0.61
Dorsomedial Striatum	51.23 (1.05)	53.39 (1.05)	0.16
Ventrolateral Striatum	52.65 (0.81)	52.78 (0.68)	0.89
Ventromedial Striatum	50.01 (1.12)	52.21 (1.02)	0.16
Nucleus Accumbens	50.62 (1.29)	52.28 (0.91)	0.31
Substantia Nigra pars Compacta	43.10 (0.88)	47.30 (0.44)	**<0.01**
Substantia Nigra pars Reticulata	47.27 (0.78)	51.19 (0.71)	**<0.01**

Table 3

Distance traveled immediately after saline or CGS21680/CPA administration (cm). A) 0.05/0.05mg/kg and B) 0.1/0.1mg/kg. Values expressed are group means with SEM in parentheses.

A

	Saline	**0.05/0.05mg/kg CGS21680/CPA**
30min post-injection	5801.8 (1026.8)	**3479.7 (685.6)***
1hr post-injection	11554.7 (1730.3)	8622.6 (1594.6)

B

	Saline	**0.1/0.1mg/kg CGS21680/CPA**
30min post-injection	6484.8 (722.9)	**3520.4 (486.3)***
1hr post-injection	14227.6 (1612.5)	**6649.6 (927.4)***

* represents statistical significance at $p<0.01$ as compared to saline control group.