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Basal Forebrain Chemogenetic Inhibition Disrupts the Superior Complex Movement Control of Goal-Tracking Rats

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Abstract

Sign- and goal-tracking behavior signifies the influence of opposed cognitive-motivational styles, with the former being characterized by a tendency for approaching and contacting reward cues, including a readiness for attending, bottom-up, to salient cues, and a relatively greater vulnerability for developing and maintaining addiction-like behaviors. We previously demonstrated that these styles also impact the cognitive-motor interactions that are taxed during traversal of dynamic surfaces, with goal-trackers (GTs) making less movement errors and falling less frequently than sign-trackers (STs). The present experiment tested the hypothesis that complex movement control in GTs, but not STs, depends on activation of the basal forebrain projection system to telencephalic regions. Chemogenetic inhibition of the basal forebrain increased movement errors and falls in GTs during traversal of a rotating zigzag rod but had no significant effect on the relatively lower performance of STs. Neurochemical evidence confirmed the efficacy of the inhibitory designer receptor exclusively activated by designer drug (DREADD). Administration of clozapine-N-oxide (CNO) had no significant effect in GTs not expressing the DREADD. These results indicate that GTs, but not STs, activate the basal forebrain projection system to mediate their relatively superior ability for complex movement control. STs may also serve as an animal model in research on the role of basal forebrain systems in aging- and Parkinson's disease-associated falls.

Keywords

basal forebrain; acetylcholine; falls; movement control; DREADD

Psychological traits and cognitive styles describe sets of cognitive-motivational-perceptual preferences and biases that impact a wide range of behavioral and cognitive processes (Sarter & Phillips, 2018). The presence of such traits typically is indicated by a behavioral test that reveals the propensity of the subject to apply such preferences or biases. Sign-trackers (STs) are animals that prefer to approach and contact Pavlovian reward cues (Meyer et al., 2012; Robinson, Yager, Cogan, & Saunders, 2014; Yager, Pitchers, Flagel, & Robinson, 2015; Yager & Robinson, 2013). This preference has been interpreted as a readiness to attribute incentive salience to such cues and, more recently, to a bias of STs for readily and reflexively deploying attention to salient cues ("bottom-up attention"). In

contrast, goal-trackers (GTs) orient to, but do not approach, reward cues, and they apply more goal-directed, or top-down, processes to respond to reward cues (Pitchers, Kane, Kim, Robinson, & Sarter, 2017; Pitchers, Phillips, Jones, Robinson, & Sarter, 2017; Pitchers, Sarter, & Robinson, 2018).

We recently demonstrated that the two opposed traits or cognitive styles indexed by sign- versus goal tracking also impact their ability to exert complex movement control. Specifically, during the traversal of a rotating zigzag rod, requiring heightened attention to the state of the rotating beam, rotating direction and rotation speed, placement of limbs, balance control, and the detection of slips and balance errors, STs were found to make more errors and fell more frequently than GTs. Moreover, GTs, but not STs, were found to skillfully time movements over particularly difficult components of the beams, thereby maintaining relatively low fall rates (Kucinski, Lustig, & Sarter, 2018).

Research on the neuronal underpinnings of the ST/GT phenotypes has focused on the contrasting reactivities of their mesolimbic dopaminergic and cortical cholinergic input systems. Specifically, the relatively superior attentional performance by GTs is mediated via a basal forebrain-cortical cholinergic projection system which, in contrast to STs, is capable of sustaining elevated levels of cholinergic activity (Koshy Cherian et al., 2017; Paolone, Angelakos, Meyer, Robinson, & Sarter, 2013). Conversely, STs, but not GTs, use elevations in mesolimbic dopaminergic signaling to approach reward cues (Pitchers, Kane, et al., 2017). Thus, STs are useful animals not only for studying vulnerabilities in the processing of reward cues (Pitchers et al., 2018), but also for the determination of the impact of relatively poor attentional control on a wide range of behavioral functions, including balance and movement. With respect to the impact of a relatively unresponsive cholinergic system on complex movement control, STs may be considered a model of the declining cholinergic-attentional functions that are associated with gait and balance control in aging humans and, more severely, in those patients with Parkinson's disease (PD) who also suffer from a propensity for falls that has been attributed to basal forebrain cholinergic losses (Bohnen et al., 2003, 2009; Brown, Shumway-Cook, & Woollacott, 1999; Kim, Muller, Bohnen, Sarter, & Lustig, 2017; LaPointe, Stierwalt, & Maitland, 2010; Springer et al., 2006; Woollacott & Shumway-Cook, 2002). Cholinergic decline is generally hypothesized to mediate a deficient processing of task and movement cues (Gritton et al., 2016), thereby impeding the cortico-striatal and striatal orchestration of movement (Sarter, Albin, Kucinski, & Lustig, 2014).

Given that GTs mediate their relatively superior attentional abilities via elevations of cholinergic activity, contrasting with a relatively lower capacity for cholinergic activation in STs (references above), the present experiment was designed to test the hypothesis that basal forebrain activation is necessary for GTs to exhibit relatively superior complex movement control. The main results indicate that chemogenetic inhibition of the basal forebrain disrupts complex movement control in GTs, but not STs, under conditions that tax cognitive-motor interactions.

Materials and Method

Subjects

Adult female and male Sprague-Dawley rats ($N = 180$; 91 females and 89 males) between 2 and 3 months of age were purchased from Envigo (Haslett, MI). Of the 91 female rats screened there were 12 GTs and 54 STs (13.19% and 59.34%, respectively), and of the 89 males there were 16 GTs and 39 STs (17.98% and 43.82%, respectively). Twenty-two GTs (12 females, 10 males) and 16 STs (eight females, eight males) were selected randomly to be used for the present experiments. For tests using the Michigan Complex Motor Control Task (MCMCT), 22 rats (six females and five males of each phenotype) received infusions of a designer receptor exclusively activated by designer drug (DREADD) construct into the basal forebrain and 6 GTs (three females and three males) received infusions of artificial cerebrospinal fluid (aCSF; details below). An additional 10 rats (three female and two male GTs; two female and three male STs) were used for microdialysis experiments and also received infusions of the DREADD construct in the basal forebrain. Rats were between 2 and 3 months of age during Pavlovian conditioned approach (PCA) screening and were between 3 and 5 months of age during MCMCT testing. Behavioral evidence obtained from some of the STs and GTs used in the present experiment, excluding effects of DREADD-related manipulations, was previously described (Kucinski et al., 2018).

Animals were individually housed in opaque single standard cages (27.70 cm $\times$ 20.30 cm) in a temperature- and humidity-controlled environment (23°C, 45%) and maintained under a 12:12 hr light/dark schedule (lights on at 7:00 a.m.). Food (Envigo Teklad rodent diet) and water were available ad libitum. PCA testing and MCMCT traversal experiments sessions were conducted during the light phase (7:00 a.m.–7:00 p.m.). All procedures were conducted in adherence with protocols approved by the Institutional Animal Care and Use Committee of the University of Michigan and in laboratories accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

PCA Screening

The purpose of this test was to determine the extent to which behavior was lever or food cup-directed. A PCA index score (below) was generated for each rat. Pavlovian training procedures were similar to those previously described (Koshy Cherian et al., 2017; Meyer et al., 2012; Pitchers, Kane, et al., 2017; Pitchers, Phillips, et al., 2017).

Apparatus and Procedures

Rats were handled daily for at least 3 days prior to screening in the Pavlovian approach test. Rats were given ~15 banana-flavored sucrose pellets (45 mg; BioServ, Flemington, NJ) in their home cages for 2 days prior to start of testing. Rats were tested in conditioning chambers (MedAssociates, Fairfax, VT; 20.5 $\times$ 24.1 cm floor area, 20.2 cm high). A food magazine with an automatic feeder that delivered sucrose pellets was located in the center of one of the walls of the chamber. Infrared photobeam breaks detected magazine entries. On either the left or right side of the magazine was a retractable lever with an LED backlight that was illuminated only when the lever extended into the chamber. Deflections of the lever were used to quantify level contacts. The beginning of a test session was signaled by the

illumination of a red house light located near the ceiling of the side of the chamber opposite to the magazine/lever. On the first day of testing (pre-training), rats were placed into the conditioning chambers and the house light was illuminated after a 5-min habituation period. Twenty-five sucrose pellets were then delivered on a VI-30 (0–60 s) schedule. The pretraining session lasted 12.5 min on average and the lever was retracted throughout the session. During this session and all subsequent PCA sessions, rats consumed all the sucrose pellets. In the next five PCA sessions, the house light was turned on after a 1-min period and rats were then presented with 25 lever-pellet pairings delivered on a VI-90 (30–150 s) schedule. The conditioned stimulus (conditional stimulus [CS]) for each trial was the extension of the illuminated lever into the chamber for 8 s. Upon retraction of the lever a sucrose pellet was delivered into the magazine. The PCA test sessions lasted 37.5 min on average.

PCA measures and classification criteria

Lever presses and magazine entries during the CS periods were used to quantify three measures of approach to compute the PCA index score: (a) Response bias was defined as the difference between lever presses and magazine entries, expressed as a proportion of the total responses [(lever presses—magazine entries)/(lever presses + magazine entries)]. (b) Latency score was calculated as the difference between the latency to approach the lever and the magazine upon CS presentation; this difference was normalized by dividing the maximum 8 s latency [(magazine latency—lever latency)/8]. (c) Probability difference was calculated as the difference between the probabilities of pressing the lever during the CS (i.e., the number of trials with a lever presses out of 25 trials) minus the probability of entering the magazine. The PCA index score was the average of the response bias score, latency score, and probability difference. The values of this score ranged from 1.0 to –1.0, with a score of 1.0 indicating approaches and contacts of the lever on every trial and a score of –1.0 indicating approaches and contacts of the magazine entry on every trial. A score of 0 indicates that lever contacts and magazines entries following CS presentation were distributed equally across trials. PCA index scores were averaged from testing Days 4 and 5 to obtain a single score that classified rats as GTs or STs. Rats were considered STs if they obtained scores ranging from 0.5 to 1.0, with scores greater than 0.4 on both days, indicating that lever-directed behavior was more than twice as frequent as food cup-directed behavior. Rats with scores ranging from –0.5 to –1.0 were classified as GTs. Rats with intermediate scores were not used in these studies.

MCMCT

The MCMCT beam traversal apparatus (for details and an illustration, see Kucinski, Paolone, Bradshaw, Albin, & Sarter, 2013) was designed to tax the ability of rats to perform attention-demanding beam traversals and correct for stepping errors while crossing a narrow square rod surface (side length: 1.59 cm). Traversal of the rod, particularly when rotating, reliably caused falls in rats with attentional deficits resulting from losses of cortical cholinergic inputs (Kucinski et al., 2013; Kucinski & Sarter, 2015). Recently, a second MCMCT apparatus was designed for compatibility with additional complex surfaces, including the zigzag rod (Kucinski et al., 2018). This apparatus featured longer (3.0 m) traversable square rods (side length 1.59 cm) comprised of aluminum tubing covered with

gray gaffer's tape for traction. In addition to a straight rod with no bends that was similar to the rod used in the first apparatus, a zigzag variation was designed to provide additional challenge during traversals. This rod includes two zigzag sections, each 0.6 m in length that began 0.6 m from either end of the rod and separated by 0.6 m of straight rod in the middle (for an illustration of the zig-zag rod, see Figure 3 in Kucinski et al., 2018). Each zigzag section includes three angled sections of varying lengths (two 10.7 cm and one 21.5 cm in length) that bended at 45° angles from the horizontal plane of the rod and which connected two straight sections (each 15 cm in length) that rested 3 cm above or below the plane the vertical plane of the rod.

The rod could be fixed stationary in either a horizontal position, so the zigzag sections protruded to the sides, requiring rats to turn left or right while traversing, or a vertical position, so the zigzag sections were angled up and down, or rotated (5 revolutions per minute [rpm]) so that rats were required to perform sideways and vertical movements while navigating over the zigzag sections. There was also a plank surface (13.3 cm width) that could be placed directly on top of the straight rod and fitted firmly in place inside edges of the support towers. The rods were rotated using a 12 VDC electric motor controlled remotely by a pulse width modulator which was able to adjust the speed of rotation (up to 10 rpm) and switch the direction of rotation. A safety net was suspended 20 cm under the beam to catch the rats during falls. Two identical end stations were situated on top the support towers at opposite ends of the beam. These stations consisted of a 30 × 25 cm platform with a 3-cm diameter copper cup embedded in the floors. Rats were given a banana pellet (45 mg per pellet; BioServ) placed inside the cups following each traversal. Each end station as surrounded by a retractable wall structure (23 cm height in raised position) to allow conversion from an open platform to an end box structure. On the wall facing the beam of each box structure a 9-cm wide opening allowed the rats access to and from the beam. The walls could be raised and lowered mechanically with a 12 VDC electric motor actuated remotely by a toggle switch.

Performance Measures

Falls, traversal time, and slips were analyzed in the same manner as previously described, with the exception that the rats' performances over each entire traversal were considered due to the longer lengths of the rods (performance measures were not prorated following falls). If, following a fall, rats were unable to resume forward traversal, the experimenter immediately assisted the rat in regaining balance on the rod, and no further slips or falls were counted until the rat regained a balanced posture and resumed forward movement. On the straight rod, because multiple falls could occur during a run, falls per run were used as a metric for fall rates. Because of the difficulty of assessing multiple falls per zigzag section, a maximum of one fall per zigzag section was scored and the percentages of zigzag sections that resulted in falls were quantified as a measure of falls on this surface. Additional analyses of performance details are described in the context of findings in the Results section. Videos of traversals were recorded with four bullet Marshall 1080-HD-DI model CV500 Series cameras (B-30/25P frame rate/59.94i) mounted on the net frame parallel to one side of the beam. The videos were converted to a single feed using a quad SDI to HDMI

multiviewer (Matrox MicroQuad, Montreal, Quebec, Canada) and viewed directly on a PC using Elgato Game Capture HD software.

Rats were first habituated with a 4-day traversal sequence (see Table 1), including trials on the plank, stationary (straight) rod, and the zigzag surface (stationary in the vertical and horizontal positions and rotating at 5 rpm). Next, the rats underwent surgery, followed by 4 weeks of recovery. The effects of clozapine-N-oxide (CNO) were then assessed using a 5-consecutive day MCMCT sequence. On the first day, all animals received vehicle injections prior to performing six runs—two on the plank surface, two on the stationary (straight) rod, and two on the straight rotating rod (cc direction; 5 rpm). Rats expressing the DREADD construct were then randomly assigned to one of two groups with equal numbers of STs and GTs (six STs and five GTs in each group; three female and three male STs per group; two female and three male GTs in one group, three female and two male GTs in the other). A separate cohort of six GTs, not expressing an active DREADD, were similarly randomly divided into two groups (three STs and three GTs in each group; two female and one male in one group, one female and two male in the other). Using a counterbalanced design, animals were tested on the straight rotating rod for two consecutive days (Days 2 and 3) followed by testing on the zigzag rotating rod for two consecutive days (Days 4 and 5). One group from each cohort received vehicle for CNO on Days 2 and 4 (see Table 1) and CNO on Days 3 and 5, the other group was given CNO and vehicle in reverse order. Injections of CNO or vehicle were given 30 to 60 min prior to MCMCT testing (drug preparation details below).

DREADD Virus Expression and CNO Administration

About two thirds of basal forebrain cholinergic neurons are noncholinergic and predominantly GABAergic (Gritti et al., 2006). As basal forebrain cholinergic and noncholinergic neurons mediate overlapping functions, including the generation of high-frequency oscillations in the cortex (Gritton et al., 2016; Howe et al., 2017; Lin, Gervasoni, & Nicolelis, 2006; Lin & Nicolelis, 2008), here the necessity of basal forebrain neuronal activity for the relatively superior complex movement control of GTs was tested by nonselectively expressing an inhibitory DREADD in cholinergic and noncholinergic neurons of the basal forebrain. In addition to this rationale, screening STs and GTs from a relatively large number of transgenic rats expressing a neuronal phenotype-specific Cre recombinase would not have been feasible. The viral vector containing plasmid pAAV-hSyn-hM4D(Gi)-mCherry was obtained from AddGene (Watertown, MA; AddGene plasmid #50475-AAV8). The adeno-associated virus (AAV), containing a double floxed muscarinic Gi-coupled-receptor, hM4D (Armbruster, Li, Pausch, Herlitze, & Roth, 2007; Roth, 2016), fused with mCherry and under the control of human synapsin promoter, was stereo-tactically infused into the basal forebrain. Rats were placed in vaporization chambers and anesthetized with 4–5% isoflurane delivered at 0.6 L/min O₂ using a SurgiVet Isotec 4 Anesthesia Vaporizer until the animals were no longer responsive to a tail pinch and exhibited no hind limb withdrawal reflex. The rats' heads were shaved using electric clippers and cleaned with a betadine scrub and alcohol pad. The animals were then mounted to a stereotaxic instrument (David Kopf Instruments) and isoflurane anesthesia was maintained at 1–3% for the remainder of the surgery. An approximately 2.5 cm incision was made down the midline of the scalp to expose the skull. The animals' body temperature was maintained at 37°C using

Deltaphase isothermal pads (Braintree Scientific). Ophthalmic ointment was used for lubrication of the eyes. To prevent hypovolemia and hemodynamic instability during prolonged surgeries, 1 mL/100g 0.9% of NaCl (s.c.) was administered. Animals also received an injection of an analgesic (Carprofen; 5.0 mg/kg; s.c) prior to surgery and once or twice daily as necessary for 48 h postoperatively. One μ L of AAV-hSyn-DIO-hM4D(Gi)-mCherry vector was infused (bolus) into the basal forebrain at two sites per hemisphere (AP -0.4 ; ML ± 2.4 ; DV: -7.6 ; AP -0.8 ; ML ± 2.9 ; DV: -8.0 mm). The injector was left in place for 8 min to minimize diffusion into the injector tract. DREADDs were activated by peripheral administration of CNO (Armbruster et al., 2007). CNO was obtained from Tocris Bioscience (Bristol, United Kingdom) and dissolved 10 mg/mL in 6% DMSO in 0.9% NaCl solution. Injections of CNO or vehicle were administered with intraperitoneal injections (i.p.) at a dose of 5.0 mg/kg 30 to 60 mins prior to MCMCT testing. As pilot data indicated effects of CNO in GTs but not STs expressing the DREADD, a separate group of GTs received infusions of aCSF (1 μ L) into the basal forebrain to assess potential effects of CNO per se (Gomez et al., 2017; Mahler & Aston-Jones, 2018).

Effects of CNO on Potassium-Evoked Cortical GABA and Acetylcholine (ACh) Release

The brief and relatively infrequent rod traversals were insufficient to demonstrate, using microdialysis, reliable increases in performance-associated neurotransmitter levels. This is in contrast with, for example, the reliably elevated increases in extracellular ACh levels in rats performing a relatively high rate of trials in a sustained attention task (Paolone et al., 2013; Paolone, Lee, & Sarter, 2012; St. Peters, Demeter, Lustig, Bruno, & Sarter, 2011). We therefore elevated cortical extracellular neurotransmitter levels by depolarizing cortical terminals via local perfusion of a relatively high concentration of potassium (100 mM). However, it is important to note that this was used as a “proof of concept” test rather than a simulation of natural activity; K⁺-evoked increases are not thought to model performance-associated elevations in neurotransmitter levels and, because K⁺-induced depolarization does not discriminate between terminals of neurons originating from the basal forebrain versus other sources, the attenuating efficacy of the inhibitory DREADD was expected to be incomplete.

Microdialysis probes with 2.0-mm long membranes were custom designed. First, two fused silica capillary tubings for inlet and outlet (75 [*i.d.*] $\times$ 150 μ m [*o.d.*]; TSP075150; Polymicro Technologies, Lisle, IL) were inserted into a 22-gauge stainless steel tubing, which served as a shaft, with a 2 mm offset at the tip for the inlet capillary. The capillary tip was ensheathed in a polyacrylonitrile membrane (20 kDa mol wt cutoff; AN69, Hospal, Lakewood, CO). The tip and the base of the membrane were sealed with epoxy resin (Loctite, Henkel). The recovery rates for these probes were 17.5% (mean; range: 10.9–31.9%) for ACh, and 16.6% (7.0–40.5%) for GABA. Final analyte concentrations were corrected by probe recovery rate. Guide cannulas (CMA12, CMA Microdialysis, Kista, Sweden) were implanted into the infralimbic region of the medial prefrontal cortex (from bregma: AP, +2.9 mm; ML, -0.6 mm; DV, 2.0 mm below skull). To prevent clogging, the cannulae were equipped with stainless steel stylets. A headstage was constructed to hold the guide cannula in place using surgical screws implanted into the skull with dental cement.

Rats were first habituated to an operant chamber (MED Associates) for 1 hr each day for 2 days. During the habituation, a tether was clamped to a hook fixed to the headstage to familiarize rats with the test conditions. On Day 3, the microdialysis session began with the removal of the stylet and the insertion of a probe. Animals were perfused at a rate of 1.0 μ l/min with aCSF (pH 7.4) containing the following (in mM): 145.00 NaCl, 2.68 KCl, 1.40 CaCl_2 , 1.01 MgSO_4 , 1.55 Na_2HPO_4 , and 0.45 NaH_2PO_4 , as well as 0.25 ascorbic acid. A relatively low concentration of neostigmine (10 nM) was also added to the aCSF perfusate to enhance the detection of ACh while having minimal effects on basal and activated ACh levels (Himmelheber, Fadel, Sarter, & Bruno, 1998). Dialysate samples were collected every 3 min, beginning 2 hr after inserting probes. After the collection of five baseline samples, potassium chloride (100 mM) was added to the perfusion medium followed by the collection of five additional samples (Herzog, Nowak, Sarter, & Bruno, 2003).

CNO was administered at the same dose used for the experiment on effects on MCMCT performance (5.0 mg/kg, i.p.; in 6% DMSO in 0.9% NaCl solution). Each DREADD-expressing rat was dialyzed twice to test CNO and vehicle administrations, with a half of the rats receiving CNO during the first session. Fifteen minutes after the injection, potassium reverse-dialysis was initiated and continued for the collection of five 3 min samples. Samples were derivatized (described below) and stored at -80°C until they were analyzed by high-performance liquid chromatography mass spectrometry (HPLC-MS).

HPLC-MS Methods

All chemicals, drugs, and reagents were purchased from Sigma-Aldrich unless otherwise noted. HPLC-grade water was purchased from VWR. d_4 -ACh and d_4 -choline (Cho) were obtained from C/D/N Isotopes. Benzoyl chloride derivatization of dialysates and internal standards (IS) was performed using a modified version of a previously described method (Song, Mabrouk, Hershey, & Kennedy, 2012). Briefly, calibration curves were generated using standards at 0, 0.1, 0.5, 1, 5, 10, and 20 nM for ACh and GABA. Internal standards were derivatized by adding 100 mM sodium carbonate buffer, followed by 2% $^{13}\text{C}_6$ benzoyl chloride in acetonitrile with 0.1% formic acid. The IS stock was then diluted 100-fold in 50% acetonitrile in water. d_4 -ACh and d_4 -Cho were spiked into the reaction mixture to a final concentration of 100 nM and 10 $\mu\text{mol/L}$, respectively. Each calibration standard or sample was mixed with 100 mM sodium carbonate buffer, 2% benzoyl chloride in acetonitrile, and IS in a 2:1:1:1 ratio.

Microdialysis samples were analyzed by a Thermo Finnigan Surveyor Plus HPLC system. Neurochemical separation was achieved with a Phenomenex Kinetex biphenyl LC column (50×2.1 mm, 1.7 μm particle size, 100 $\text{\AA}$ pore size). Mobile phase A consisted of 10 mM ammonium formate and 0.15% (vol/vol) formic acid in water. Mobile phase B was acetonitrile. The mobile phase gradient was as follows: initial, 0% B; 0.1 min, 10% B; 0.12 min, 10% B; 2.3 min, 20% B; 3.7 min, 50% B; 4.0 min, 80% B; 4.5 min, 100% B; 5.0 min 100% B; 6.5 min, 0% B. The flow rate was 200 $\mu\text{l/min}$, and the sample injection volume was 7 μl . The autosampler and column were maintained at ambient temperature throughout the analysis.

A Thermo Finnigan TSQ Quantum Ultra triple quadrupole mass spectrometer operating in positive mode was used for detection. Electrospray ionization (ESI) voltage was 3.5 kV, and heated ESI probe (HESI-I) was set at 300°C. Capillary temperature was 350°C, and sheath gas, aux gas, and ion sweep gas were maintained at 25, 15, and 0 arb, respectively. The intercycle delay was 200 ms. Automated peak integration was performed using Thermo XCalibur QuanBrowser Version 2.1. All peaks were visually inspected to ensure proper integration. Calibration curves were constructed based on peak area ratio ($P_{\text{analyte}}/P_{\text{IS}}$) versus concentrations by linear regression. Note that absolute ACh levels reported herein differ from those reported previously (Koshy Cherian et al., 2017) because of recent modifications and refinements of our analytical HPLC-MS methods.

Histology

Following completion of behavioral testing, animals were deeply anesthetized using sodium pentobarbital (270 mg/kg; i.p.) and transcardially perfused with phosphate buffer solution (PBS) followed by 4% paraformaldehyde in 0.15 M sodium-phosphate buffer with 15% picric acid, pH 7.4. Brains were removed and postfixed for 2–6 hrs at 4°C and then rinsed in 0.1 M PBS and stored in 10% sucrose in PBS solution overnight, followed by 30% sucrose in PBS solution the following day and allowed to sink. Coronal sections (40 μ) were sliced using a freezing microtome (CM 2000R; Leica) and stored in 0.1 M PBS or antifreeze solution until additional processing. Parallel sections were processed for the immunohistochemical visualization of mCherry stained sections. mCherry immunostaining was performed using a primary antibody (ab16453; Abcam; rabbit antimCherry) and Vectastain Elite ABC kit (PK-6100; Vector Laboratories). Sections were first rinsed three times for 5 min each in Tris-Triton (1:10 dilution), pH 7.4, and then incubated in 3% peroxide for 10 min. They were rinsed again in Tris-Triton stock solution three times for 5 min each before being incubated in the primary antibody (rabbit antim-Cherry made in 1% Normal Donkey Serum, 1:500) overnight at room temperature. The next day, the sections were rinsed three times for 5 min each in Tris-Triton and then incubated in the biotinylated secondary antibody (biotinylated donkey antirabbit in 1% Normal Donkey Serum; 17 1:500) for 30 min. They were then rinsed three times for 5 min each and then incubated with ABC elite (1:1000) for 30 min. The sections were again rinsed three times for 5 min in the Tris-Triton before being developed for 10 min using a Vector Laboratories diaminobenzidine (DAB) Peroxidase Substrate Kit (SK-4100; made in distilled water). Sections were rinsed in Tris-Triton three times for 5 min each before being mounted onto gelatin-coated slides and allowed to dry overnight. The following day, slides were dehydrated in an ascending alcohol series (70%, 90%, and 100%) and defatted in xylene before coverslipping. mCherry stained slides were imaged using a Leica DM400B digital microscope and a Zeiss LM 700 confocal microscope.

Experimental Design and Statistical Analysis

The present experiments were designed to test the hypothesis that chemogenetic inhibition of the basal forebrain of GTs results in ST-like impairments. For PCA testing, approach responses (response probability, number of contacts, and latency) were analyzed with repeated-measures analyses of variance (ANOVAs) with phenotype (STs, GTs) as the between groups factor and training day (1–5) as the within-subject factor. One- or two-way

ANOVAs were used to compare PCA scores between GTs and STs and sexes. MCMCT performance measures (falls, traversal time, and slips) were compared between GTs and STs and sexes using within-subjects repeated measures ANOVAs. To assess the effects of CNO on zigzag traversal performance, CNO (or vehicle) was used as a repeated measures within-subjects factor. Assumptions underlying the statistical model were assessed. In cases of violation of the sphericity assumption, Huynh-Feldt-corrected F values, along with uncorrected degrees of freedom, are given. Post hoc analyses for all within-subjects comparisons and additional analyses were performed using the t test, least significant difference (LSD) test, or χ^2 test when applicable. Potassium-evoked increases in, and CNO-induced attenuation of, neurotransmitter levels ($n = 5/\text{phenotype}$) were analyzed using the nonparametric sign test. Statistical analyses were performed using the SPSS for Windows (Version 17.0: SPSS). Alpha was set at 0.05. Exact p values are reported as recommended previously (Greenwald, Gonzalez, Harris, & Guthrie, 1996; Sarter & Fritschy, 2008). Effect sizes (Cohen's d) were computed for major results (Cohen, 1988).

Results

PCA-Based Screening of STs and GTs

A total of 22 GTs (12 females) and 16 STs (8 females) were tested on the MCMCT or underwent microdialysis. The PCA scores of these STs and GTs differed significantly ($F(1, 34) = 1384.55, p > .001$; PCA scores: GTs: -0.77 ± 0.03 ; STs: $+0.79 \pm 0.03$). PCA scores were not affected by sex (main effect sex and phenotype interaction both $F < 0.95$, both $p > .33$).

STs and GTs were familiarized with the 3-m long MCMCT apparatus, including practice on the rotating zigzag rod, and then received bilateral infusions of the DREADD construct or, in a separate group of GTs, of aCSF, into the basal forebrain. Four weeks postsurgery, rats underwent a 4-day test sequence in which the effects of vehicle or CNO-induced inhibition of basal forebrain neurons were assessed (see the Methods section for justification of expression of the DREADD in cholinergic and noncholinergic neurons; see also Table 1).

On the first day of postsurgery testing, rats were refamiliarized with traversing the plank, stationary, and rotating straight rod (cc direction, 5 rpm; two trials per condition). Separate analyses of rotating rod (Postsurgery Days 2 and 3) and rotating zigzag beam (Days 4 and 5) performance, when rats were administered CNO or vehicle in accordance with a counterbalanced sequence, were conducted to determine effects of phenotype, CNO treatment and sex.

Rotating Rod Performance

Compared with the effects of vehicle, administration of CNO increased falls, slips, and traversal time (alpha set at 0.05/3; main effects of CNO: all three $F(1, 18) > 12.28$, all $p < .003$; falls: vehicle: 0.63 ± 0.15 falls per run, CNO: 0.97 ± 0.18 ; Figure 1a–c). Although inspection of Figure 1 suggests that the detrimental effects of CNO were greater in STs, the effects of CNO and phenotype did not interact with respect to the three measures of performance (main effects of phenotype and interactions: all $F < 2.91$, all $p > .11$). Falls,

slips, and traversal time were significantly correlated, in STs and GTs, and following vehicle as well as CNO treatment (all $R^2 > 0.85$, all $p < .001$; Figure 1d–f).

Similar to previously reported sex effects (Kucinski, de Jong, & Sarter, 2017), male rats fell more frequently than female rats, $F(1, 18) = 8.68$, $p = .009$ (males: 1.24 ± 0.29 falls per run, females: 0.43 ± 0.06); they committed significantly more slips, $F(1, 18) = 13.13$, $p = .002$ (males: 9.77 ± 1.94 slips per run, females: 3.04 ± 0.50); and they traversed the rotating rod more slowly, $F(1, 18) = 15.43$, $p = .01$ (males: 18.97 ± 2.84 s, females: 8.49 ± 0.81 s). However, these effects of sex did not interact with the effects of phenotype and CNO (all $p > .11$). To further explore this main effect of sex, males weighed more than females ($F(1, 18) = 404.11$, $p < .001$; males: 451.70 ± 8.48 g; females: 291.58 ± 3.00 g). However, as also observed previously, body weights of male or female rats did not correlate with falls, slips or traversal time (all $p > .19$).

Zigzag Beam Performance

Rats traversed the zigzag rod on Days 4 and 5. If a fall occurred at one of the two zigzag sections (Figure 2a), rats were placed back on the rod near the straight section separating the two zigzag parts. For this reason, falls were calculated not over runs per day but over the number of zigzag sections traversed per day (12 sections/day).

Administration of CNO increased the number of falls—main effect CNO: $F(1, 16) = 18.97$, $p < .001$; vehicle: $17.50 \pm 3.31\%$, falls CNO: $35.00 \pm 4.22\%$). The effects of CNO and phenotype on falls interacted significantly ($F(1, 16) = 5.41$, $p = .03$; Figure 2b; main effects of phenotype, sex and interactions: all $F < 3.21$; all $p > .09$). Multiple comparisons (Figure 2b) indicated that when given vehicle, STs fell significantly more frequently than GTs, $F(1, 19) = 6.69$, $p = .019$, and that CNO significantly increased falls in GTs, $t(9) = 37.55$, $p < .001$; Cohen's d : 1.62 but not STs, $t(9) = 1.43$, $p = .26$. Falls from the zigzag beam were not affected by sex, and sex did not interact with the effects of phenotype or CNO (all $F < 3.20$, all $p > .09$).

The greater vulnerability of STs for falls and the selective effect of CNO in GTs was not paralleled by significant main effects of CNO (both $F < 5.98$, both $p > .05$), and interactions with phenotype, on slips and traversal speed (both $F < 2.94$, both $p > .10$; Figure 2c and 2d). There was a trend for a main effect of CNO on slips, $F(1, 16) = 5.98$, $p = .050$ (slips vehicle: 5.12 ± 0.75 slips, CNO: 7.21 ± 1.17 ; Figure 2b). Males had more slips than females, $F(1, 16) = 11.19$, $p = .004$ (males: 14.84 ± 2.05 slips; females: 9.87 ± 3.29 slips), but this effect of sex did not interact with any other factor (all $F < 1.81$, all $p > .19$). Likewise, zigzag beam traversal speed was not affected by CNO or phenotype (main effects and interactions (all $F < 2.94$, all $p > .10$; Figure 2f). As before, males were generally slower than females, $F(1, 16) = 9.44$, $p = .007$ (males: 25.22 ± 3.37 s, females: 14.74 ± 1.89 s) but the effects of sex did not interact with any other factor (all $F < 2.32$, all $p > .14$).

CON increased zigzag entrance pauses.—We previously described evidence indicating that GTs avoided entries onto the angled parts when they were at steep angles or moving toward such a riskier state (Kucinski et al., 2018). For this analysis, we classified a steep angle at the time of entrance (61° – 90°) as a relatively riskier state of the angled part.

Moreover, if an angled part, facing up or down, or rotated (30°/s) toward a steeper angle, this was also classified as a “difficult approach.” We inspected the effects of CNO in GTs during difficult approaches. However, no effects were determined, perhaps indicating a “ceiling” effect for this category of approaches. In contrast, an analysis of “easy approaches,” defined as the initial part of the zigzag being at a flat or at a medium angle indicated a significantly greater number of falls in GTs when given CNO, $t(106) = 6.69$, $p = .01$; vehicle: $3.45 \pm 2.42\%$ falls; CNO: $18.37 \pm 5.59\%$. When given CNO, GTs increased their pause time prior to entering an angled part, and this effect was associated with falls (pause time in the absence of falls ($n = 40$ cases): 1.04 ± 0.20 s, pause times with subsequent falls ($n = 9$ cases): 2.56 ± 0.53 s, $t(48) = 9.63$, $p = .003$). Pause times nearing 3 s corresponded with almost 90° of rotation, suggesting that under the influence of CNO, the ability of GTs to time their entry onto the angled part while still at a relatively easy state was disrupted.

No Effects of CNO in GTs Not Expressing a DREADD

To assess potential effects of CNO-derived clozapine (Gomez et al., 2017; Mahler & Aston-Jones, 2018), CNO was tested in a separate group of GTs which had received infusions of aCSF into the basal forebrain. This test of effects of CNO was restricted to GTs because CNO exclusively impaired GTs’ performance on the zigzag rod.

CNO per se neither affected straight rotating rod nor zigzag rod performance. Concerning rotating rod performance, CNO administration did not increase falls when compared with the effects of vehicle for CNO in these rats, $F(1, 4) = 0.20$, $p = .68$ (falls—vehicle: 0.72 ± 0.15 falls per run, CNO: 0.83 ± 0.25). Slips and traversal time were also unaffected by CNO (both $F < 0.26$, both $p > .64$). Once again, males overall committed more falls than females, $F(1, 4) = 10.87$, $p = .03$ (falls—males: 1.11 ± 0.15 falls per run, females: 0.45 ± 0.14) but this effect did not interact with effects of CNO, $F(1, 4) = 0.20$, $p = .68$. Likewise, CNO did not affect fall rates on the zigzag rod, $F(1, 4) = 0.10$, $p = .77$ (falls—vehicle: $13.89 \pm 7.95\%$, CNO: $19.44 \pm 7.95\%$). For this condition, there was no sex effect and no interaction between effects of sex and CNO (both $F < 1.76$, both $p > .25$).

DREADD Reporter Expression in the Basal Forebrain and Neurochemical Efficacy

The inhibitory DREADD was expressed in cholinergic as well as noncholinergic neurons in the basal forebrain of rats (see Methods for justification; Figures 3 and 4). Within the target region of the virus infusions, the nucleus basalis of Meynert (nbM) and the substantia innominata, approximately 60–80% of cholinergic neurons were transfected with the DREADD virus, accounting roughly for one third of mCherry-positive neurons (see Figure 4).

Initial attempts to collect cortical dialysates during MCMCT runs did not yield reliable increases in recovered neurotransmitter levels, probably due to the relatively short runs over the rotating rod (10–15 s) separated by 1–2 min periods during which rats remained in the end stations and until they self-initiated beam traversal after lowering of the station walls. In contrast to, for example, the relatively high density of trials during sustained attention performance that reliably increases ACh levels (e.g., St. Peters et al., 2011), rod traversals may be too brief and infrequent to yield sufficient increases in extracellular neurotransmitter

levels that can be recovered by microdialysis probes with 10–20% recovery rates. To generate a measure of the neurochemical efficacy of CNO, we therefore elevated cortical neurotransmitter levels by reverse-dialyzing potassium (Herzog et al., 2003; Moore, Stuckman, Sarter, & Bruno, 1996) and assessed the efficacy of CNO to attenuate such elevations.

Five rats per phenotype (STs: two females, three males; GTs: three females, two males) received infusions of the DREADD virus in the basal forebrain and were prepared for cortical microdialysis while freely moving (see Methods). The effects of K^+ added to the microdialysis perfusion medium were determined in the presence and absence of CNO (5.0 mg/kg). Similar to our prior finding, basal extracellular GABA levels did not differ between the phenotypes (sign test; $z = 0.44$, $p = .65$; Figure 5a). K^+ perfusion increased GABA levels in both phenotypes (both $z > 2.20$, both $p < .03$), and CNO robustly attenuated these increases, reaching significance in STs (STs: $z = 2.23$, $P = 0.03$; GTs: $z = 1.34$, $p = .17$). Also reproducing previous results, basal extracellular ACh levels did not differ between GTs and STs ($z = 0.45$, $p = .65$) and perfusion with K^+ elevated ACh levels in GTs ($z = 2.23$, $p = .03$), but not STs ($z = 1.34$, $p = .18$), likely reflecting the choline transporter dysfunction that characterizes STs (Koshy Cherian et al., 2017). CNO administration attenuated the K^+ -evoked increases in GTs (Figure 5b) but the effect did not reach significance ($z = 0.44$, $p = .65$). Together, these results indicate a more robust efficacy of CNO on GABA, consistent with the relatively larger population of noncholinergic and thus likely GABAergic neurons transfected by the DREADD construct.

Discussion

Previous studies indicated that GTs, relatively to STs, exhibit superior attentional control (for a description of this construct, see Sarter & Paolone, 2011), which is mediated via a basal forebrain cholinergic system capable of supporting sustained elevations of activity. In STs, dysregulation of cellular mechanisms that control the capacity of the neuronal choline transporter limits sustained increases in cholinergic signaling, therefore allowing a dopaminergically mediated approach behavior to predominate over cholinergic control of attention-evoking cues (Koshy Cherian et al., 2017; Paolone et al., 2013; Pitchers et al., 2015, 2018; Pitchers, Kane, et al., 2017; Pitchers, Phillips, et al., 2017; Sarter & Phillips, 2018). Furthermore, we previously demonstrated that the impact of traits that are signified by sign- versus goal-tracking extends to complex movement control, using the same behavioral apparatus employed in the present experiment (Kucinski et al., 2018). Here we tested the hypothesis that the superior complex movement control in GTs depends on activity in the basal forebrain. The main result indicates that when such control is taxed at a relatively high degree, as is the case when traversing the rotating zigzag rod, inhibition of the basal forebrain significantly and, in terms of effect size, extensively, impairs the performance of GTs, but not STs.

In the present experiment the inhibitory DREADD was expressed nonselectively in cholinergic as well as noncholinergic neurons of the basal forebrain. This was due in part to the practical infeasibility of generating a sufficiently large colony of transgenic rats to screen adequate numbers of STs and GTs. Moreover, this approach also reflected the view that in

order to evaluate the necessity of basal forebrain neuronal activity for behavior, insulated manipulations of just one neuronal genotype would ignore evidence indicating the cholinergic and noncholinergic neurons of the basal forebrain act in concert to mediate the detection of cues and the degree of top-down control (Sarter & Bruno, 2002; Tingley, Alexander, Quinn, Chiba, & Nitz, 2015). Specifically, just as cholinergic neurons are necessary for the detection of attention-demanding cues (Gritton et al., 2016; McGaughy, Kaiser, & Sarter, 1996; Sarter, Lustig, Berry, et al., 2016), noncholinergic neurons encode the behavioral salience of such cues (Avila & Lin, 2014; Lin & Nicolelis, 2008). Furthermore, just as cue-associated cholinergic signaling induces cortical oscillations in the gamma range and theta-gamma coupling to “broadcast: cue information across larger brain regions” (Howe et al., 2017), synchronized activation of noncholinergic neurons in the basal forebrain induces oscillatory activity in the cortex, including activity in the gamma range (Anacleit et al., 2015; Lin et al., 2006). Thus, assessing the necessity of basal forebrain activation demanded inhibition of cholinergic as well as noncholinergic neurons.

As indicated in Methods, the brief and relatively infrequent rod traversals were insufficient to demonstrate reliable increases in performance-associated neurotransmitter levels using microdialysis. Thus, to demonstrate the neurochemical efficacy of the inhibitory DREADD, we elevated cortical extracellular neurotransmitter levels by depolarizing cortical terminals via local perfusion of potassium. K^+ robustly increased cortical GABA levels in both phenotypes and, reproducing a prior finding, ACh levels in GTs only (Koshy Cherian et al., 2017). Activation of the inhibitory DREADD attenuated the elevated GABA levels in both phenotypes but the effect did not reach significance in GTs, reflecting the variability of the effects of reverse dialysis of K^+ on release (Figure 4a). For the same reason, the suppression of the elevated ACh levels in GTs did not reach significance. These data indicate the limitations of using K^+ elevation to show the efficacy of an inhibitory DREADD on release. Future research will need to explore new avenues to show elevated neurotransmitter levels in rats traversing the 3-m long beams, to then demonstrate more conclusive neurochemical efficacy of DREADD activation.

Although the demonstration of the neurochemical efficacy of DREADD activation focused on the prelimbic region, consistent with prior evidence showing that STs exhibit a blunted ACh response to the presentation of a cocaine cue (Pitchers, Kane, et al., 2017), the present effects of CNO likely reflected suppression of cholinergic and noncholinergic neurotransmission across a more widespread telencephalic area, not including, however, the vertical limb of the horizontal band and thus projections to the hippocampus. In the context of complex movement control, it may be speculated that suppression of basal forebrain-cortical processing of task- and movement cues also disrupts the cortico-striatal transfer of such information, thereby depriving the striatum from input normally used to orchestrate movement selection and sequencing (Sarter et al., 2014).

As shown before (Kucinski et al., 2018), during traversal of the particularly difficult rotating zigzag rod STs fell more frequently than GTs. Administration of CNO increased falls significantly in GTs but not STs. STs fell while traversing approximately 25% of zigzag sections (note again that there are two sections per run; see Figure 2a) and thus it seems unlikely that the relatively high fall propensity of STs at baseline prevented the

demonstration of potential CNO effects. Rather, activation of the basal forebrain by GTs may have supported their relatively superior performance on the zigzag rod while such activation, at least with respect to cholinergic neurons, may have remained limited or was absent in STs (Koshy Cherian et al., 2017). This interpretation is consistent with prior data showing that GTs, but not STs, activate their cholinergic system in the presence of a cue that was paired with cocaine administration (Pitchers, Kane, et al., 2017), and that loss of basal forebrain cholinergic neurons disrupts cue-evoked cocaine seeking in GTs but not STs (Pitchers, Phillips, et al., 2017). Together, these results indicate that GTs recruit their basal forebrain for attentional cue processing while the basal forebrain of STs, at least the cholinergic component, is less capable of supporting cue detection and attentional control (see also Pitchers et al., 2018).

In contrast to rotating zigzag rod traversal, traversal of the straight rotating rod was not differentially affected by the administration of CNO. It seems likely that following several practice runs, straight rod traversal rapidly transforms to a relatively habitual behavior, with stable and fluent stepping and movement patterns supporting the relatively low fall rates in both phenotypes (Figure 1a). Thus, straight rotating rod traversal may not depend significantly on basal forebrain activation, as also indicated by the absence of effects of basal forebrain cholinergic lesions on such behavior (Kucinski et al., 2013). In contrast, GTs use timing to enter the rotating zigzag sections when in, or rotating toward, a relatively “easy” state, involving presumably the detection of angles and rotation states and thus relatively high levels of attentional control (Kucinski et al., 2018). Therefore, traversing rotating zigzag sections likely required a more robust activation of basal forebrain circuitry significantly than straight rotating rod traversal, favoring GTs and supporting their superior performance, and thus rendering GTs more vulnerable to the effects of chemogenetic inhibition of this brain region.

We previously demonstrated in unscreened rats that combined basal forebrain cholinergic and striatal dopamine losses, but not solely cholinergic or dopaminergic losses, reproduced the falls seen in PD fallers with such combined losses (Kucinski et al., 2013, 2017; Kucinski & Sarter, 2016; Sarter et al., 2014). In the current experiment, nonselective suppression of the BF induced falls in GTs but not STs. It is not known whether nonselective basal forebrain lesions alone would cause falls, or whether selective cholinergic lesions alone would cause falls if tested in GTs only. However, selective cholinergic lesions in GTs were found to disrupt the response of GTs, but not STs, to a discriminative cue predicting the availability of cocaine (Pitchers, Phillips, et al., 2017). Although this prior evidence concerns a different behavioral domain, the interpretation is consistent with that of the current effects of suppression of basal forebrain inhibition on falls in GTs. In both cases, disruption of basal forebrain function attenuates the attentional supervision of a complex behavior that depends on the effective use of contextual and task cues. Acute and nonselective disruption of basal forebrain activity in GTs, as opposed to the long-term impact of selective cholinergic losses, may sufficiently disrupt complex movement control that relies on the incorporation of highly specific task cues, such as the state and direction of rotation of the zigzag sections.

In the absence of any basal forebrain manipulations, STs may serve as precursor model of the rat model for falls. To reiterate, the rat model is based on combined losses of about 50% of the cholinergic projections of the basal forebrain and dopamine depletions restricted to the mediodorsal caudate nucleus (Kucinski et al., 2013). STs have a limited capacity for elevating cholinergic activity because of their unresponsive choline transporter (Koshy Cherian et al., 2017). The presence and impact of this neuromarker for STs is unlikely restricted to a single region in the brain and thus may also limit the capacity of striatal interneurons to integrate cortico-striatal and dopaminergic functions (Aldrin-Kirk et al., 2018; Aoki, Liu, Zucca, Zucca, & Wickens, 2015; Augustin, Chancey, & Lovinger, 2018; Deffains & Bergman, 2015; Kosillo, Zhang, Threlfell, & Cragg, 2016; Maurice et al., 2015; Zucca, Zucca, Nakano, Aoki, & Wickens, 2018). Although these issues remain to be investigated, it is therefore plausible to conceptualize the relatively attenuated attentional-motor capacities of STs (see also Kucinski et al., 2018) in accordance with the same theoretical framework as employed for falls in PD (Sarter et al., 2014).

In GTs not expressing the DREADD, CNO had no effects on straight rotating rod or rotating zigzag rod performance. Given the proposed conversion rate for CNO to clozapine (Gomez et al., 2017), our dose of CNO equated to the administration of 0.05 mg/kg clozapine. This dose is six times smaller than the highest tested in rats performing a working memory task and that still remained ineffective (Gemperle, McAllister, & Olpe, 2003), and 50 times smaller than a dose found to impair attention performance (see also Mahler & Aston-Jones, 2018; Martinez & Sarter, 2008). Moreover, clozapine would increase cortical ACh levels (Ichikawa, Dai, O'Laughlin, Fowler, & Meltzer, 2002; Parada, Hernandez, Puig de Parada, Rada, & Murzi, 1997), contrasting with the attenuating effects of CNO. Thus, it seems unlikely that the effects of CNO were confounded by CNO-derived clozapine, and the absence of effects of CNO in control rats confirms this view.

In conclusion, the results from the present experiment indicate that complex movement control in GTs, but not STs, depends on activation of the basal forebrain. This evidence is consistent with the prior conceptualization of GTs as preferring a relatively “cold” top-down analysis of the instrumental significance of cues (Pitchers et al., 2018) and indicates a key role of the basal forebrain for the execution of this cognitive style (Sarter & Phillips, 2018). The current evidence also suggests sign-tracking in humans (Joyner, Gearhardt, & Fligel, 2018) and the presence of a cognitive style that is characterized by relatively low levels of attentional control, such as in humans expressing a low-capacity choline transporter (Sarter, Lustig, Blakely, & Koshy Cherian, 2016). The present results predict that these phenotypes are associated with deficits in complex movement control (Arnold et al., 2018; Rinne et al., 2018) and an elevated risk for falls when traversing dynamic surfaces (Allcock et al., 2009; Bohnen et al., 2009; Brown et al., 1999; LaPointe et al., 2010; O'Halloran et al., 2011; Woollacott & Shumway-Cook, 2002). Finally, as STs continue to be considered to a useful model for studying the role of a psychological trait for addiction vulnerability (Pitchers et al., 2018; Robinson, Carr, & Kawa, 2018; Singer, Fadanelli, Kawa, & Robinson, 2018), the present results raise the possibility that complex movement control deficits are present in vulnerable subjects, and that such deficits may be associated with comorbid risks for age-related falls and movement disorders.

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Figure 1.

Effects of administration of clozapine-N-oxide (CNO) on rotating rod performance ($n = 11$ goal-trackers [GTs] and 11 sign-trackers [STs] with inhibitory designer receptor exclusively activated by designer drug (DREADD) in basal forebrain, five males and six females per group). CNO administration resulted in more falls (a), more slips (b) and slower traversal time (c). In rats not expressing the DREADD, CNO was ineffective (see the Results section). CNO did not affect any performance measures in GTs not expressing the DREADD (see the Results section). Falls, slips and traversal time were highly correlated, irrespective of the treatment (CNO vs. vehicle for CNO; d–f; red (light gray) filled symbols: STs; blue (dark gray) filled symbols: GTs; the upper right blue (dark gray)-red (light gray) symbol in Panel f depicts two overlapping data points from a ST and a GT treated with CNO). See the online article for the color version of this figure.

Figure 2.

(a) Illustration of the 3-m long zigzag beam. Note that the beam consisted of two zigzag sections separated by a horizontal straight part; the number of falls was calculated based on the number of sections traversed (one beam run = two sections). Falls occurred primarily from the first two angled parts of each zigzag section. (b) The effects of clozapine-N-oxide (CNO)-induced inhibition of basal forebrain neurons on falls interacted with phenotype, reflecting that, following vehicle treatment, sign-trackers (STs) committed more falls than goal-trackers (GTs), and that CNO treatment increased fall rates in GTs only ($n = 10$ GTs and 10 STs; four males and six females per group). This interaction was not associated with significant effects on slips (c) and traversal time (d) see the Results section for analyses of variance; post hoc comparisons: * $p < .01$, ** $p < .001$. CNO had no effects in GTs not expressing the designer receptor exclusively activated by designer drug (DREADD; see Results). See the online article for the color version of this figure.

Figure 3.

Representative designer receptor exclusively activated by designer drug (DREADD) virus expression spaces (DAB chromogenic stain for m-Cherry) in the basal forebrain of a female goal-tracker (GT; a–d) and a male sign-tracker (ST; e–h). The frames in a, c, e, and g (scales: 1,000 μ m) are magnified b, d, f, and h (scales: 500 μ m), respectively. In the anterior basal forebrain (a, b, e, and f), globus pallidus (GP) neurons widely expressed the virus, including the cholinergic neurons that are organized along the medial wall of the GP and in the region transitioning to the ventral pallidum (VP). As can be seen in [b and f, labeled neurons were also found in the bed nucleus of the stria terminalis (BNST). More ventral parts of the basal forebrain, including the preoptic region and diagonal band, did not express the DREADD virus. In the posterior basal forebrain (c, d, g, and h), virus expression centered around the ventro-medial GP where the majority of magnocellular cholinergic neurons (nucleus basalis of Meynert [nbM]) are located and more variably extended to the more ventral region containing the smaller cholinergic neurons of the substantia innominata (SI). Virus expression did not extend to the vertical limb of the diagonal band that projects to hippocampus. I and J show labeled axons and varicosities in the middle layers in the somatosensory cortex (scales: 100 μ m), reflecting the widespread cortical projection pattern of basal forebrain cholinergic and noncholinergic neurons. Systematic differences in the expression of the virus between male and female rats, and between STs and GTs were not observed, consistent with the lack of differential effects of DREADD activation in male and female rats, and in STs and GTs when assessed on the relatively less demanding straight rotating rod. CPu = caudate-putamen. See the online article for the color version of this figure.

Figure 4.

Confocal photomicrographs of nucleus basalis of Meynert (nbM) neurons expressing the mCherry reporter of the designer receptor exclusively activated by designer drug (DREADD) virus (red [dark gray]) and the vesicular acetylcholine transporter (VAcHT; green [light gray]). (a) depicts a relatively low-power photomicrograph of the dorsal part of the nbM showing double-labeled neurons (see arrows) as well as numerous neurons expressing mCherry only. Double-labeled neurons were verified using confocal microscopy. (b) higher power photomicrograph of five VAcHT- and mCherry-positive neurons in the nbM. Approximately 60–80% of cholinergic neurons were transfected with the DREADD virus, accounting roughly for one third of mCherry-positive neurons (100 µm-scales are indicated in a and b). See the online article for the color version of this figure.

Figure 5.

Effects of the administration of clozapine-N-oxide (CNO) in rats ($n = 5$ goal-trackers [GTs] and 5 sign-trackers [STs]; 2 male and 3 female GTs, 3 male and 2 female STs) expressing an inhibitory designer receptor exclusively activated by designer drug (DREADD) in the basal forebrain on increases in cortical extracellular GABA (a) and acetylcholine (ACh; b) levels produced by reverse microdialysis of potassium (boxes and whiskers indicate the 25th–75th percentile and the range of values, respectively; lines indicate the median value). Potassium increased GABA levels in both phenotypes and CNO attenuated these elevated levels, although the effect reached significance in STs only (nonparametric sign-test; * $p < .05$). Reproducing a prior result (Koshy Cherian et al., 2017), potassium increased ACh levels only in GTs. CNO insignificantly reduced the elevated ACh levels in GTs. See the online article for the color version of this figure.

Table 1

Michigan Complex Motor Control Task Conditions

Day	Beam	Rotating (speed)	Number of trials
Presurgery			
1	Plank	n/a	6
2	Straight rod	no	6
3	Horizontal zig-zag	no	3
	Vertical zig-zag	no	3
4	Zig-zag	5 rpm	6
Postsurgery			
1 (vehicle)	Plank	n/a	2
	Straight rod	no	2
	Straight rod	5 rpm	2
2 (CNO or vehicle)	Straight rod	10 rpm	6
3 (CNO or vehicle)	Straight rod	10 rpm	6
4 (CNO or vehicle)	Zig-zag	5 rpm	3
5 (CNO or vehicle)	Zig-zag	5 rpm	3

Note. CNO = clozapine-N-oxide; RPM = revolutions per minute.