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Activation of Neurotensin Receptor Type 1 Attenuates Locomotor Activity

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Abstract

Intracerebroventricular administration of neurotensin (NT) suppresses locomotor activity. However, the brain regions that mediate the locomotor depressant effect of NT and receptor subtype-specific mechanisms involved are unclear. Using a brain-penetrating, selective NT receptor type 1 (NTS1) agonist PD149163, we investigated the effect of systemic and brain region-specific NTS1 activation on locomotor activity. Systemic administration of PD149163 attenuated the locomotor activity of C57BL/6J mice both in a novel environment and in their homecage. However, mice developed tolerance to the hypolocomotor effect of PD149163 (0.1 mg/kg, *i.p.*). Since NTS1 is known to modulate dopaminergic signaling, we examined whether PD149163 blocks dopamine receptor-mediated hyperactivity. Pretreatment with PD149163 (0.1 or 0.05 mg/kg, *i.p.*) inhibited D2R agonist bromocriptine (8 mg/kg, *i.p.*)-mediated hyperactivity. D1R agonist SKF81297 (8 mg/kg, *i.p.*)-induced hyperlocomotion was only inhibited by 0.1 mg/kg of PD149163. Since the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) have been implicated in the behavioral effects of NT, we examined whether microinjection of PD149163 into these regions reduces locomotion. Microinjection of PD149163 (2 pmol) into the NAc, but not the mPFC suppressed locomotor activity. In summary, our results indicate that systemic and intra-NAc activation of NTS1 is sufficient to reduce locomotion and NTS1 activation inhibits D2R-

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6. Conflict of Interest

The authors declare no biomedical financial interests or potential conflicts of interest.

mediated hyperactivity. Our study will be helpful to identify pharmacological factors and a possible therapeutic window for NTS1-targeted therapies for movement disorders.

Keywords

Nucleus accumbens; NTS1; PD149163; Neurotensin; Locomotor activity; D2R

1. Introduction

Neurotensin (NT) is a tridecapeptide that functions as a hormone in the periphery and as a neurotransmitter in the central nervous system (CNS) (Carraway and Leeman, 1975; Mendez et al., 1997; Wang and Evers, 1999). In the CNS, NT modulates dopamine signaling which is hypothesized to be the mechanism behind the locomotor effects of NT (Binder et al., 2001; Nemeroff et al., 1982). Intracerebroventricular (*i.c.v.*) administration of NT suppresses locomotion, but NT has no effect on locomotion when given peripherally (Elliott et al., 1986; Lambert et al., 1995; Meisenberg and Simmons, 1985; Nemeroff et al., 1977). Since NT does not cross the blood brain barrier and is quickly degraded by peptidases (Boules et al., 2013), NT-induced hypolocomotion is likely mediated by NT receptors in the CNS. However, the brain regions involved in NT-mediated hypolocomotion are not clear.

Microinjection studies have identified specific brain regions that may play an important role in the locomotor effects of NT. Interestingly, microinjection of NT into the ventral tegmental area (VTA) promotes locomotion, while NT injection into the nucleus accumbens (NAc) blocks the hyperlocomotor effect induced by NT administration into the VTA (Kalivas et al., 1982). Furthermore, intra-NAc administration of NT attenuates dopamine-mediated hyperactivity (Kalivas et al., 1984). Since *i.c.v.* administration of NT induces hypolocomotion and microinjection of NT into the NAc suppresses dopamine-mediated hyperactivity, NT receptor activation in the NAc may determine its overall effect on locomotion. Although the NAc is associated with reward behaviors, several studies point to a role of the NAc in movement (Humphries and Prescott, 2010; Pennartz et al., 1994). Anatomically, the NAc can be considered a limbic-motor interface (Mogenson et al., 1980). The NAc receives input from limbic regions like the amygdala and hippocampus. In addition, dopaminergic neurons project to the NAc primarily from the VTA. In turn, efferents from the NAc project to brain regions associated with generating movement such as the ventral pallidum and brainstem nuclei (Humphries and Prescott, 2010). Studies have shown that firing patterns of neuronal subpopulations in the NAc predict the direction and intensity of movement (Nicola, 2007; Taha et al., 2007). Thus, NT could reduce locomotion by activating NT receptors in the NAc, which may alter the activity of the resident efferent medium spiny neurons.

Currently, there are three known receptors (NTS1, NTS2, and NTS3/sortilin) through which the biological effects of NT are mediated (Chalon et al., 1996; Mazella et al., 1998; Tanaka et al., 1990). NTS1 and NTS2 are G-protein-coupled receptors, while NTS3 is a predominantly intracellular, single transmembrane receptor implicated in protein sorting, cell death and inflammation (Mazella and Vincent, 2006). NTS1 has the highest affinity for

NT and functional studies suggest that the hypolocomotor effect of NT could be mediated through NTS1. NTS1 KO mice are hyperactive and systemic administration of a blood brain barrier permeable, selective NTS1 agonist, PD149163 inhibits amphetamine-mediated hyperlocomotion in rats (Feifel et al., 2008; Liang et al., 2010). Depending on the dose, *i.c.v.* administration of NT induces hypolocomotion in wild-type, but not NTS1 KO mice (Remaury et al., 2002).

One possible mechanism by which NT may induce hypolocomotion is through modulation of dopamine neurotransmission. NTS1 is thought to inhibit dopamine transmission through an interaction with dopamine D2 receptors (D2R) (Binder et al., 2001). D2R activity appears to be positively correlated with locomotor activity given that D2R KO mice are hypoactive and D2R antagonists suppress locomotion (Kelly et al., 1998; Klinker et al., 2013). Therefore, one mechanism by which NT may reduce locomotor activity could be through inhibition of D2R function.

Here we sought to determine whether NTS1 activation reduces locomotion in mice by systemically administering a blood brain barrier permeable, selective NTS1 agonist, PD149163. Since there is a well-established interaction between NTS1 and D2R, we wanted next to determine if PD149163 can inhibit D2R-mediated hyperactivity. Furthermore, as there is evidence to suggest that the NAc may play a major role in NT-mediated hypolocomotion, we examined whether microinjection of PD149163 into the NAc reduces locomotor activity.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (6 weeks old, Jackson Laboratories, Bar Harbor, ME) were grouped housed (4-5 mice per group) in standard Plexiglas cages under a 12 h light/dark cycle with lights on at 6:00 AM. Food and water was provided *ad libitum*. Mice were used for the behavioral studies between 8-16 weeks of age. Animal care and handling procedures were approved by the Mayo Clinic Institutional Animal Care and Use Committees in accordance with National Institutes of Health guidelines.

All mice were naïve to the open-field tests and used only once in the same behavioral test. However, since the grip strength test does not interfere open-field or rotarod tests, we used some mice twice to minimize the number of mice. We used the two groups of the same mice for open-field or rotarod experiments before or after the grip strength tests with one-week interval between experiments. For the first group, mice were used to examine recovery from the hypo-locomotor effect of 0.1 mg/kg of PD149163 in the open-field ($n=14$, one mouse was excluded due to a sensor malfunction). One week after, the same mice were used to examine the effect of 0.5 mg/kg of PD149163 1 h after the injection (*i.p.*) on grip strength ($n=14$). For the second group, mice were used for the effect of PD149163 30 min after the injection (*i.p.*) on grip strength ($n=16$). One week after the test, 23 naïve mice were added to the group of mice, totaling 39 mice, which were randomly distributed to examine the effect of PD149163 on ataxia ($n=17$) and tolerance to 0.5 mg/kg of PD149163 in the open field ($n=22$). For all the other experiments, mice were naïve to PD149163. Tolerance to the

hypothermic effects of PD149163 was evaluated by taking body temperature measurements before and after mice were placed in the open-field in locomotor tolerance experiments.

2.2. Drugs

PD149163 tetrahydrochloride hydrate (PD149163) and the dopamine D1 receptor (D1R) agonist, ($\pm$)-6-Chloro-PB hydrobromide (SKF-81297), were purchased from Sigma-Aldrich (St. Louis, MS, USA). PD149163 and SKF-81297 were dissolved in 0.9% saline. For 0.05 and 0.1 mg/kg doses of PD149163, PD149163 was administered at 0.01 mg/mL. A dilution of 0.02 mg/mL of PD149163 was used for the 0.5 mg/kg dose. SKF-81297 was administered at a dose of 8 mg/kg at a concentration of 0.8 mg/mL. The D2R agonist, bromocriptine mesylate (bromocriptine) was purchased from TOCRIS Bioscience (Park Ellisville, MO, USA). Bromocriptine was dissolved in 0.9% saline, 5% DMSO and 5% Cremophor. Bromocriptine was administered at a dose of 8 mg/kg at a concentration of 0.5 mg/mL.

2.3. Open-field

For all open-field experiments, spontaneous locomotor activity was measured during the light phase in open-field chambers (27 cm x 27 cm) equipped with two sets of infrared photobeams to record X-Y ambulatory movements at a 50 ms resolution (Med Associates, Lafayette, IN). The chambers were located in brightly lit (500 lux), sound-attenuating cubicles. All mice were allowed to habituate to the room for 1 h prior to locomotor measurements. Activity was quantified as horizontal distance traveled (cm).

2.3.1. Dose-dependent effect of systemic PD149163 administration on locomotor activity—To examine the systemic effect of PD149163 on locomotor activity in a novel environment, mice were divided into four groups which received saline, 0.05, 0.1 or 0.5 mg/kg of PD149163 (*i.p.*). Mice were returned to their homecages then placed in the open-field 30 min later. Their activity was recorded for 1 h. Total distance traveled in 1 h and distance traveled per 10 min was quantified.

2.3.2. Recovery from the hypolocomotor effect of systemic PD149163 administration—To assess how long it takes mice to recover from the locomotor depressant effect of PD149163, mice were placed in the open-field 30 min after saline or PD149163 (0.1 mg/kg, *i.p.*). Locomotor activity was recorded for 15 min and mice were placed back in their homecage at the end of the session. Mice were then continually placed in the open-field every hour for 15 min for 3.5 h. Total distance traveled was determined for each 15 min session. Mice were placed in the open-field for 15 min sessions every hour to slow habituation of the mice to the chambers.

2.3.3. Tolerance to the hypolocomotor effect of PD149163—To determine if mice develop tolerance to the hypolocomotor effect of PD149163, mice were divided into three treatment groups (control, acute and chronic) for each tolerance experiment. We performed four experiments with separate groups of mice that were all naïve to the open-field. We investigated whether mice develop tolerance to three or seven days of once-daily repeated PD149163 administration, using two different doses of PD149163 (0.1, 0.5 mg/kg, *i.p.*). Each mouse was injected once per day with saline or PD149163 in the afternoon. Control

mice received only saline. Acutely treated mice received saline, but on the test day (day 3 or day 7) were administered PD149163. Chronically treated mice received PD149163 each day. On the test day, mice received saline or PD149163 and were placed back in their homecages for 30 min. Mice were then placed in the open-field and locomotor activity was recorded for 1 h. The total horizontal distance traveled in 1 h was quantified.

To assess whether mice also develop tolerance to the hypothermic effect of PD149163, we recorded the body temperature of the mice during the tolerance experiments. Body temperature was measured using a rectal thermometer connected to a Physiomex monitor (Columbus Instruments, OH, USA). Basal body temperatures were recorded. In addition, body temperatures were measured 30 and 90 min after drug administration, which was just before placement in the open-field and at removal from the open-field, respectively.

2.3.4. Effect of PD149163 on dopamine receptor-mediated hyperactivity—To examine the effect of PD149163 on D2R-mediated hyperactivity, PD149163 (0.05, 0.1 mg/kg, *i.p.*) or saline was administered 15 min before injection of a D2R agonist, bromocriptine (8 mg/kg, *i.p.*) or vehicle (0.9% saline, 5% DMSO, 5% Cremaphor, *i.p.*). Mice were placed in the open-field 3 h after the last injection and locomotor activity was recorded for 1 h. Locomotor activity was not assessed until 3 h after bromocriptine or vehicle administration since it has been previously reported that bromocriptine exerts a slow-acting hyperlocomotor effect in mice (Dogrul and Yesilyurt, 1999). We also examined if PD149163 has the same effect on D2R-mediated hyperactivity when given after bromocriptine. In this experiment, mice received bromocriptine or vehicle, then saline or PD149163 (0.05 mg/kg, *i.p.*) 2.5 h later. Mice were placed in the open-field 30 min after the last injection (3 h after the first injection, bromocriptine or vehicle) and locomotor activity was assessed for 1 h.

To examine the effect of PD149163 on D1R-mediated hyperactivity, PD149163 (0.05, 0.1 mg/kg, *i.p.*) or saline was administered 15 min before a D1R-selective agonist, SKF-81297 (8 mg/kg, *i.p.*). Mice were placed in the open-field 15 min after SKF-81297 administration since it has been shown to rapidly induce hyperactivity (Zhuang et al., 2001). Locomotor activity was assessed for 1 h. For all of the experiments, the total horizontal distance traveled was quantified.

2.3.5. Microinjection of PD149163—Mice first underwent surgery for guide cannulae implantation. Mice were anesthetized with ketamine/xylazine (100 mg/kg and 15 mg/kg, *i.p.*) and were placed in a digital stereotaxic frame (Model 1900, David Kopf Instruments, Tujunga, CA). Guide cannulae (26 Ga double guide, Plastics One) were bilaterally implanted into the NAc (anteroposterior (AP): 1.3 mm; mediolateral (ML): ± 1.0 mm from bregma; dorsoventral (DV): -2.25 mm from dura) or mPFC (AP: 1.9 mm; ML: ± 0.25 mm from bregma; DV: -1.0 mm from dura). Guide cannulae were held into position by a skull screw and dental cement. Dummy cannulae were inserted to keep the guide cannulae free of tissue and debris during recovery. Following surgery mice were singly housed. Mice were given 6-7 days to recover after surgery.

Dummy cannulae were removed and the injectors (33 Ga double internal, Plastics One) were inserted. Injectors extended beyond the guide cannulae 2.0 mm to target the NAc and 0.5 mm to target the mPFC. PD149163 (10 μ M in 0.9 % saline) or saline was microinjected at a flow rate of 0.1 μ l/min for 2 min, resulting in 2 pmol of PD149163 in 0.2 μ l per hemisphere. The dose of PD149163 was chosen based upon reported CNS microinjection studies using PD149163 (Buhler et al., 2005; Norman et al., 2008). Injectors were left in place for an additional minute before removing. Mice were placed in open-field chambers 30 min after microinjection and locomotor activity was measured for 1 h. Using a separate group of mice, we also examined the effect of microinjection of PD149163 at the same dose and flow rate into the NAc on locomotor activity in the open-field immediately following the microinjection. This additional experiment was carried out to confirm our observations that PD149163 does not immediately reduce locomotor activity after microinjection, which is why we chose to examine the activity of mice 30 min after administration into the NAc or mPFC. For all the microinjection experiments we quantified distance traveled per 10 min in the open-field.

Following the experiment Fast Green dye was microinjected using the same volume and flow rate to visualize the injection site. Brains were removed 30 min after dye microinjection and fixed in 4% paraformaldehyde in 1 $\times$ PBS for 24 h. Brains were cryoprotected by incubation in 30% sucrose then sliced using the cryostat. Tissue was subsequently stained with hematoxylin and eosin. Mice with injectors in the wrong location were excluded from the study.

2.4. Homecage activity

Mice were singly housed and given two weeks to adjust to the housing conditions. The light cycle remained the same with lights on at 6 AM and off at 6 PM. Homecage activity was monitored using overhead infrared sensors connected to the Clocklab system (Coulbourn Instruments, Whitehall, PA). To assess the effect of NTS1 activation on homecage activity, PD149163 (0.5 mg/kg, *i.p.*) or saline was administered for three days just before lights off at approximately 5 PM. Since mice are nocturnal, we considered each 24 h period to begin at lights off (6 PM), and end at 6 PM the next day. Baseline (day 0), post-injection (days 1-3) and recovery (day 4) activities were recorded. Therefore, saline or PD149163 was administered at the end of days 0-2. Activity duration and intensity were summed over early dark phase (6 PM-12 AM, 18-0 h), late dark phase (12 AM- 6 AM, 0-6 h) and light phase (6 AM-6 PM, 6-18 h) for each 24 h period. For data acquisition and actogram analysis, activity bouts were defined as a period of continuous activity (regardless of duration) separated by at least 5 min of activity quiescence.

2.5. Grip strength

Mice were injected with saline or PD149163 (0.5 mg/kg, *i.p.*) and grip strength was quantified 30 min after administration. Using a separate group of mice, grip strength was also quantified 1 h after injection of 0.5 mg/kg of PD149163. A grip strength meter from Bioseb (Chaville, France) was used for these experiments. Grip strength was quantified by placing all four limbs on a wire grid (100 $\times$ 100 mm, 45° angle) that was attached to a force gage. An experimenter blind to the treatments subsequently pulled the mouse down until it

released its grasp from the grid. Each mouse was tested three times consecutively and the average force in grams was reported for each animal.

2.6. Rotarod

A mouse rotarod treadmill (Ugo Basile, Italy) rotating at a fixed speed of 20 rpm was used to measure motor incoordination (ataxia). On day 1, mice were trained to stay on the rotarod for 180 s. Mice that required more than 8 trials to stay on the rotarod were excluded from the study. To determine the time course of recovery from the ataxic effect of PD149163 (0.1, 0.5 mg/kg, *i.p.*), mice were placed on the rotarod every 30 min for 5 h after drug administration. The latency to fall off the rotarod was recorded at each timepoint. Mice were placed back in the homecage once they fell or after they had remained on the rotarod for 180 s.

2.7. Statistical analysis

Data are presented as mean $\pm$ s.e.m. (standard error of the mean). Grip strength data were analyzed by unpaired two-tailed *t*-tests (Prism 5 software, Graphpad). Open-field experiments were analyzed by one-way ANOVA or two-way repeated measures ANOVA (SigmaPlot 12.0, Systat Software). Homecage activity, rotarod and body temperature experiments were also analyzed by two-way repeated measures ANOVA. ANOVA was followed by Tukey *post-hoc* tests for individual comparisons where appropriate. Results were considered statistically significant when $p < 0.05$.

3. Results

3.1. Systemic NTS1 activation suppresses locomotion of mice in a novel environment

3.1.1. PD149163 dose-dependently reduces locomotor activity of mice naïve to the open-field—First, we examined the dose-dependent effect of PD149163 on the locomotor activity of mice naïve to the open-field. PD149163 (0.05 to 0.5 mg/kg, *i.p.*) or saline as a control was administered to mice (**Fig. 1A**). One-way ANOVA indicated a significant effect of treatment [$F_{(3,31)} = 60.14, p < 0.0001, n = 8-9$]. Tukey *post-hoc* analyses revealed the dose-dependent effects of PD149163 on total distance traveled. Interestingly, even at the lowest dose (0.05 mg/kg) PD149163 suppressed locomotor activity significantly while higher doses (0.1, 0.5 mg/kg) of PD149163 further reduced locomotion. There was no difference in the locomotor activity of the mice treated with 0.1 mg/kg in comparison 0.5 mg/kg of PD149163. We also analyzed the distance traveled per 10 min by the mice in the open-field after PD149163 or saline administration (**Fig. 1B**). Two-way repeated measures ANOVA showed effects of treatment [$F_{(3,155)} = 60.14, p < 0.001$], time [$F_{(5,155)} = 63.16, p < 0.001$], and an interaction [$F_{(15,155)} = 8.46, p < 0.001$]. The higher doses (0.1, 0.5 mg/kg) of PD149163 resulted in reduced activity relative to saline- and 0.05 mg/kg-treated mice at all timepoints, as shown by Tukey *post-hoc* analyses. Tukey *post-hoc* analyses also revealed that 0.5 mg/kg of PD149163 had a greater hypolocomotor effect relative to 0.1 mg/kg-treated mice during the initial 10 min in the open-field. Furthermore, the lowest dose of PD149163 did not significantly reduce locomotor activity until 60 min after administration. These results indicate that systemic activation of NTS1 induces

hypolocomotion in C57BL/6J mice in a novel environment in a dose- and time-dependent manner.

3.1.2. PD149163 transiently reduces locomotion—To demonstrate that mice recover from the locomotor depressant effect of PD149163, mice were placed in the open-field after saline or PD149163 (0.1 mg/kg, *i.p.*) for 15 min every hour for 3.5 h (**Fig. 1C**). Two-way repeated measures ANOVA showed an effect of treatment [$F_{(1,33)} = 33.0, p < 0.001, n = 6-7$], time [$F_{(3,33)} = 12.8, p < 0.001$] and an interaction [$F_{(3,33)} = 8.0, p < 0.001$]. Tukey *post-hoc* tests showed that PD149163 reduced the locomotor activity of mice relative to saline-treated mice after 30 min, 1.5 h and 2.5 h. However, the PD149163-treated mice displayed similar locomotor activity in the open-field relative to the saline-treated mice when examined 3.5 h after injection. Thus, the hypolocomotor effect of PD149163 after systemic administration is transient since mice were found to recover from the locomotor depressant effect of 0.1 mg/kg of PD149163 after 3.5 h.

3.1.3. Mice develop tolerance to the hypolocomotor effect of PD149163—Since it has been reported that rodents can develop tolerance to the hypolocomotor effect of NT (Rinkel et al., 1983), we examined the effect of chronic PD149163 administration on locomotion. Mice in the chronic treatment group were given 0.1 or 0.5 mg/kg of PD149163 (*i.p.*) daily for three or seven days. The control group received saline each day. The acutely-treated group received saline until the test day (day 3 or day 7) where mice received PD149163. We assessed locomotor activity 30 min after the last injection on the test day. Mice developed tolerance to the hypolocomotor effect of 0.1 mg/kg of PD149163 after seven repeated administrations (**Fig. 2A**). One-way ANOVA showed a significant effect of treatment [$F_{(2,15)} = 27.3, p < 0.0001, n = 6$]. Tukey *post-hoc* tests revealed that a single or acute administration of 0.1 mg/kg of PD149163 suppressed the activity of mice in the open-field relative to saline-treated mice. However, after seven days of 0.1 mg/kg of PD149163, mice showed similar locomotion in the open-field relative to the saline-treated mice. To determine if mice develop tolerance to the hypolocomotor effect of 0.1 mg/kg of PD149163 after fewer treatments, mice received three daily injections of saline or PD149163 (**Fig. 2B**). One-way ANOVA indicated a significant effect of treatment [$F_{(2,14)} = 10.0, p = 0.002, n = 5-6$]. Again, acute treatment with 0.1 mg/kg of PD149163 reduced locomotion, but three daily injections resulted in similar locomotor activity relative to the saline-treated mice, as determined by Tukey *post-hoc* tests. Therefore, mice develop tolerance to the hypolocomotor effect of just three repeated administrations of 0.1 mg/kg of PD149163.

We also investigated whether mice develop tolerance to three repeated injections of a higher dose of PD149163 (0.5 mg/kg, *i.p.*) (**Fig. 2C**). One-way ANOVA showed a significant effect of treatment [$F_{(2,21)} = 62.3, p < 0.0001, n = 8$]. Tukey *post-hoc* tests revealed that both acute and three repeated injections of 0.5 mg/kg of PD149163 similarly reduced locomotor activity relative to saline-treated mice. We then examined whether mice develop tolerance after seven daily administrations of 0.5 mg/kg of PD149163 (**Fig. 2D**). There was a significant effect of PD149163 treatment as shown by one-way ANOVA [$F_{(2,19)} = 56.3, p < 0.0001, n = 6-8$]. Again Tukey *post-hoc* tests revealed that both acute and seven repeated injections of 0.5 mg/kg of PD149163 similarly reduced locomotor activity relative to saline-

treated mice. Together this shows that mice develop tolerance to the hypolocomotor effect of 0.1 mg/kg of PD149163 after three or seven daily treatments, but not with three or seven repeated treatments of 0.5 mg/kg of PD149163.

Since NT is known to induce hypothermia which may contribute to the locomotor effect of PD149163, we measured the body temperature of the mice during the tolerance experiments. Body temperature was measured at baseline, 30 min (before placement in the open-field) and 90 min (upon removal from the open-field) after PD149163 administration. Mice exhibited tolerance to the hypothermic effect of PD149163 after seven administrations of 0.1 mg/kg of PD149163 (**Supplementary Fig. 1A**). Two-way repeated measures ANOVA revealed a significant effect of treatment [$F_{(2,30)} = 8.0, p = 0.004, n = 6$], time [$F_{(2,30)} = 25.6, p < 0.001$] and an interaction [$F_{(4,30)} = 23.4, p < 0.001$]. Only acute treatment with 0.1 mg/kg of PD149163 was observed to reduce body temperature 90 min after administration, as determined by Tukey *post-hoc* tests. Mice in the different treatment groups had similar body temperatures at baseline and hypothermia was not observed 30 min after PD149163 treatment. Mice also developed tolerance to the hypothermic effect of PD149163 after three administrations of 0.1 mg/kg of PD149163 (**Supplementary Fig. 1B**). Two-way repeated measures ANOVA revealed a significant effect of treatment [$F_{(2,28)} = 14.0, p < 0.001, n = 5-6$], time [$F_{(14,28)} = 12.0, p < 0.001$] and no interaction [$F_{(4,28)} = 23.4, p = 0.059$]. Acute PD149163 treatment significantly reduced body temperature relative to control and chronically-treated mice (Tukey *post-hoc* tests). Unlike what was observed with tolerance to the locomotor effect of a higher 0.5 mg/kg dose of PD149163, mice developed tolerance to the hypothermic effect of seven repeated injections of 0.5 mg/kg of PD149163 (**Supplementary Fig. 1C**). Two-way repeated measures ANOVA revealed a significant effect of treatment [$F_{(2,38)} = 21.2, p < 0.001, n = 6-8$], time [$F_{(2,38)} = 53.9, p < 0.001$] and an interaction [$F_{(4,38)} = 20.9, p < 0.001$]. Tukey *post-hoc* tests revealed that acute treatment with 0.5 mg/kg of PD149163 induced hypothermia 30 and 90 min after administration. However, 0.5 mg/kg of PD149163 produced hypothermia in chronically treated mice only after 90 min and the effect was significantly reduced relative to the acutely treated mice (Tukey *post-hoc* tests). It is interesting that seven days of injections with 0.5 mg/kg of PD149163 did not induce tolerance to the hypolocomotor effect, but did induce tolerance to the hypothermic effect. In addition, no hypothermic effect was apparent 30 min after administration of 0.1 mg/kg of PD149163, unlike what was previously observed in the open-field experiment (**Fig. 1B**). Together, this suggests that the locomotor and hypothermic effects of PD149163 may be dissociable.

3.2. NTS1 activation suppresses homecage activity of mice

Since exposure to a novel environment is known to induce hyperactivity, we determined if NTS1 activation also reduces basal homecage activity. Homecage activity was assessed over five days. Baseline activity is reported as day 0. During days 0-2, saline or PD149163 (0.5 mg/kg, *i.p.*) was administered before lights off (17 h or 5 PM) which was just before the next nocturnal active phase. Therefore, we recorded post-injection activity as days 1-3, and recovery activity as day 4. Activity distribution patterns were depicted with actograms. Activity was plotted over 24 h beginning at 18 h or 6 PM. As depicted in representative actograms (**Fig. 3A and B**), PD149163 treatment appeared to suppress homecage activity

during the earlier half of the dark phase. Therefore, we analyzed homecage activity data during three time periods: early dark phase (18-0 h), late dark phase (0-6 h) and light phase (6-18 h). Analysis of early dark phase activity duration by two-way repeated measures ANOVA revealed a significant effect of treatment [$F_{(1,40)} = 14.6, p = 0.003, n = 5-7$], day [$F_{(4,40)} = 35.6, p < 0.001$] and an interaction [**Fig. 3C**; $F_{(4,40)} = 4.7, p = 0.003$]. Tukey *post-hoc* tests showed no difference in early dark phase activity duration between the two groups of mice at day 0 or baseline. Treatment with 0.5 mg/kg of PD149163 decreased early dark phase activity duration relative to saline-treated mice on days 1-3. Upon cessation of injections on day 4, early dark phase activity duration returned to saline-treated levels of activity. Early dark phase activity duration was reduced on days 1-3 relative to day 0 in saline-treated mice, which may be explained by injection and handling stress. PD149163 treatment did not affect late dark phase or light phase activity duration as determined by two-way repeated measures ANOVA (**Fig. 3D and E**). Analysis of early dark phase activity intensity revealed a significant effect of treatment [**Fig. 3F**; $F_{(1,40)} = 5.6, p = 0.04$] and day [$F_{(4,40)} = 24.2, p < 0.001$], but no interaction [$F_{(4,40)} = 1.9, p = 0.127$]. Mice treated with 0.5 mg/kg of PD149163 had on average reduced early dark phase activity intensity (263.8 ± 49.4 , mean $\pm$ s.e.m.) relative to saline-treated mice (444.6 ± 58.4 , mean $\pm$ s.e.m.). PD149163 treatment did not affect late dark phase or light phase activity intensity as determined by two-way repeated measures ANOVA (**Fig. 3G and H**). No effect of PD149163 treatment on late dark phase or light phase activity suggests that the effect of PD149163 disappeared within 6 h since PD149163 was administered just before lights off. Taken together, these results indicate that systemic NTS1 activation reduces locomotion of mice in the homecage.

3.3. NTS1 activation impairs rotarod performance, but does not affect grip strength

3.3.1. PD149163 impairs rotarod performance—Next, we asked if PD149163 impairs locomotion when mice are forced to ambulate on the rotarod. PD149163 at both doses (0.1 mg/kg, 0.5 mg/kg) impaired the ability of the mice to stay on the rotarod (**Fig. 4A**). Two-way repeated measures ANOVA showed an effect of time [$F_{(10,150)} = 19.5, p < 0.001, n = 8-9$], treatment [$F_{(1,150)} = 142.4, p < 0.001$] and an interaction [$F_{(10,150)} = 10.0, p < 0.001$]. Tukey *post-hoc* tests revealed that both treatments significantly impaired rotarod performance 30 min after administration relative to their baseline performance. Mice recovered from the motor-impairing effect of 0.1 mg/kg of PD149163 after 2.5 h as determined by Tukey *post-hoc* test. However, more than 5 h was required for mice to recover from the motor-impairing effect of 0.5 mg/kg of PD149163, which is in line with our findings showing that homecage activity was reduced during the first 6 h after administration of 0.5 mg/kg of PD149163. Overall this suggests that systemic NTS1 activation transiently impairs performance on the rotarod.

3.3.2. PD149163 does not affect grip strength—Since reduced neuromuscular function may be attributed to PD149163-induced ataxia on the rotarod, we assessed neuromuscular function by measuring grip strength at 30 min (**Fig. 4B**) and 1 h (**Fig. 4C**) after PD149163 (0.5 mg/kg, *i.p.*) treatment. We found that PD149163 had no effect on grip strength 30 min [$t_{(14)} = 0.1, p = 0.936, n = 8$] or 1 h [$t_{(12)} = 0.4, p = 0.722, n = 7$] after administration relative to saline-treated mice. These data indicate that PD149163 does not alter neuromuscular function.

3.4. NTS1 activation blocks dopamine receptor-mediated hyperlocomotion

To determine if NTS1 activation inhibits D2R-mediated hyperlocomotion, mice were pretreated with PD149163 15 min prior to administration of a D2R-selective agonist bromocriptine (8 mg/kg, *i.p.*). We assessed locomotor activity 3 h after the last injection since bromocriptine has been reported to exert a peak effect 3-4 h after administration (Dogrul and Yesilyurt, 1999). One-way ANOVA revealed a significant effect of treatment [Fig. 5A; $F_{(3, 25)} = 31.1, p < 0.001, n = 6-8$]. Individual comparisons by Tukey *post-hoc* tests indicated that bromocriptine increased locomotor activity while both a low (0.05 mg/kg) and a moderate dose (0.1 mg/kg) of PD149163 blocked bromocriptine-induced hyperlocomotion. There was no difference in locomotor activity of mice treated with 0.05 mg/kg PD149163 and bromocriptine in comparison to mice treated with 0.1 mg/kg of PD149163 and bromocriptine. Together, these data suggest that PD149163 potentially blocks D2R-mediated hyperlocomotion.

Next, we examined if NTS1 activation inhibits D1R-mediated hyperlocomotion. Mice were pretreated with PD149163 15 min prior to D1R-selective agonist SKF-81297 (8 mg/kg, *i.p.*) since this dose of SKF-81297 has been previously reported to induce hyperlocomotion in mice (Zhuang et al., 2001). Mice were then placed in the open-field 15 min after the last injection since SKF-81297 rapidly induces hyperactivity (Zhuang et al., 2001). One-way ANOVA revealed a significant effect of treatment [Fig. 5B; $F_{(3, 20)} = 44.8, p < 0.0001, n = 5-7$]. Tukey *post-hoc* comparisons indicated that SKF-81297 induced hyperlocomotion while only the moderate dose of PD149163 (0.1 mg/kg) blocked the hyperactivity. Taken together, our data indicate that while pretreatment with PD149163 may block D1R-mediated hyperlocomotion, PD149163 more potently blocks D2R-induced locomotion.

Since PD149163 was given at different timepoints in the D2R and D1R agonist experiments, which may explain the differences in effect of PD149163 on dopamine receptor-mediated hyperactivity, we investigated the effect of PD149163 given after bromocriptine on D2R-induced hyperactivity. PD149163 (0.05 mg/kg, *i.p.*) was given 2.5 h after bromocriptine (8 mg/kg, *i.p.*) or vehicle. Locomotor activity was then assessed 30 min after the last injection. One-way ANOVA revealed a significant effect of treatment [Fig. 5C; $F_{(2, 18)} = 59.0, p < 0.0001, n = 6-8$]. Bromocriptine-induced hyperactivity and PD149163 given after bromocriptine still reduced bromocriptine mediated hyperactivity, as determined by Tukey *post-hoc* tests. Overall, this further supports that PD149163 may selectively inhibit D2R-mediated hyperactivity.

3.5. NTS1 activation in the NAc, but not the mPFC is sufficient to reduce locomotor activity

Postsynaptic inhibition of D2R function in the striatum may partially explain mechanistically how an NTS1 agonist suppresses locomotion (Binder et al., 2001). Moreover, NTS1 is highly expressed postsynaptically in the ventral striatum which consists of the NAc (Pickel et al., 2001). Thus, we examined if PD149163 microinjection into the NAc reduces locomotion. PD149163 was microinjected into the NAc to result in 2 pmol bilaterally. Mice were returned to their homecages for a period of rest after 3 min of restraint and then were placed in the open-field 30 min after the start of the microinjection. We chose to place mice in the open-field 30 min after microinjection since PD149163 induced

hypolocomotion only after more than 10 min when mice were placed in the open-field immediately after microinjection of PD149163 at the same dose and flow rate into the NAc (**Supplementary Fig. 2A and B**). The observed placement of the injectors into the NAc is depicted in **Fig. 6A**. Microinjection of PD149163 into the NAc suppressed locomotion as indicated by a significant effect of treatment by two-way repeated measures ANOVA [**Fig. 6B**; $F_{(1, 50)} = 13.2, p = 0.005, n = 6$]. Two-way repeated measures ANOVA also revealed a significant effect of time [$F_{(5, 50)} = 21.1, p < 0.001$] and an interaction [$F_{(5, 50)} = 20.1, p < 0.001$]. Tukey *post-hoc* tests showed that microinjection of PD149163 into the NAc reduced locomotor activity in the open-field relative to saline-treated mice during the first 30 min in the open-field. Postsynaptically, NTS1 is also expressed in the mPFC and thus we wanted to determine if activation of NTS1 in a different brain region also reduces locomotion. The observed injector locations in the mPFC are depicted in **Fig. 6C**. Using the same injection paradigm, PD149163 microinjection into the mPFC was found to have no effect on locomotion by two-way repeated measures ANOVA (**Fig. 6D**). In conclusion, NTS1 activation in the NAc, but not the mPFC is sufficient to induce hypolocomotion.

4. Discussion

In this study, we found that systemic administration of a NTS1-selective agonist PD149163 reduces both activity in a novel environment (open-field) and basal homecage activity in mice. We also observed dose-dependent effects of PD149163 on locomotor activity with 0.5 mg/kg exerting a maximal hypolocomotor effect and 0.05 mg/kg displaying a nearly half-maximal effect. Hypolocomotor effects were apparent 30 min after 0.1 and 0.5 mg/kg of PD149163. However, 0.05 mg/kg of PD149163 did not significantly reduce locomotion in the open-field until 60 min after administration. The slow-acting effect of PD149163 on locomotion may be attributed to the pharmacokinetics and pharmacodynamics of PD149163. Although the NT analog PD149163 has been engineered to readily cross the blood brain barrier, it is a large molecule, which would be expected to slow its accumulation in the brain (Wustrow et al., 1995). It has also been proposed that PD149163 may act as a pro-drug which could contribute to its slow-acting effects (Boules et al., 2006). Furthermore, PD149163 has been shown to specifically activate the G protein-coupled receptor, NTS1 (Petrie et al., 2004). Thus, the slow metabotropic pharmacodynamics of PD149163 may also explain the time course of the effects on locomotor activity in mice. The long-lasting effect of PD149163 may be attributed to the increased resistance of PD149163 to enzymatic degradation (Wustrow et al., 1995).

Mice did develop tolerance to the hypolocomotor effect of 0.1 mg/kg of PD149163 after three repeated injections. This is interesting since rodents have been reported to develop rapid tolerance to some effects of NT agonists (such as hypothermia), but do not develop tolerance to the antipsychotic-like effects of NT (Boules et al., 2003; Feifel et al., 2008; Hadden et al., 2005). There is also interest in developing drugs targeting the NT system for the treatment of addiction, especially for alcohol use disorders (Lee et al., 2010). In future studies, it will be important to explore whether repeated administration of PD149163 in mice reduces alcohol drinking or schizophrenia-like behavior with minimal locomotor side effects. We also found that mice did not develop tolerance to seven repeated administrations of 0.5 mg/kg of PD149163. It is possible that this may have been due to floor effects related

to using such a high dose of PD149163. Contrary to our findings, a study reported a trend for PD149163 (1 mg/kg, *i.p.*) to reduce locomotion in rats and after eight injections PD149163 significantly increased locomotor activity relative to saline-treated rats (Feifel et al., 2008). The difference between these studies may be due to several factors including species differences and the dose regimen. One possible explanation is that Feifel et al. (2008) habituated rats to the open-field chambers and measured activity counts rather than total distance traveled. It is possible that more frequent injections and a longer treatment regimen of PD149163 may result in tolerance to the locomotor effects of higher doses in C57BL/6J mice. Higher doses of PD149163 may be useful for the treatment of hyperactivity and therefore tolerance to locomotor effects of PD149163 is something that will have to be carefully considered.

PD149163 also transiently impaired the performance of mice on the rotarod. However, we observed that PD149163 did not reduce grip strength which suggests that impaired rotarod performance is not due to a loss of neuromuscular function. Our results showing that NTS1 activation impairs rotarod performance is supported by other findings demonstrating that non-selective NT analogs reduce the latency to fall from the rotarod in wild-type, but not NTS1 KO mice (Lee et al., 2010; Pettibone et al., 2002). Further studies are necessary to determine if PD149163-mediated impairment in rotarod performance is due to a general decrease in locomotion or impaired motor coordination.

Our microinjection studies revealed that NTS1 activation in the NAc, but not the mPFC is sufficient to induce hypolocomotion. Although NTS1 is found on cortical pyramidal neurons and interneurons in the mPFC, our data suggest that NTS1 activation in the mPFC does not play a major role in NT-mediated hypolocomotion (Petrie et al., 2005). Supporting our findings, microinjection of various doses of NT into the mPFC has also been shown to have no effect on basal locomotor activity of rats (Radcliffe and Erwin, 1996). Disrupting the activity of neurons in the NAc pharmacologically or by lesioning has been reported to alter locomotor activity (Donzanti and Uretsky, 1983; Fink and Smith, 1980; Ikemoto, 2002; Kelly et al., 1976; Morgenstern et al., 1984; Pycock and Horton, 1979; Schwarting and Huston, 1996). Moreover, microinjection of NT into the NAc has been shown to inhibit dopamine- and psychostimulant-mediated hyperactivity (Ervin et al., 1981; Kalivas et al., 1984; Robledo et al., 1993; Steinberg et al., 1994). Injection of NT into the dorsal striatum does not alter amphetamine-induced hyperactivity which is in agreement with the lack of postsynaptic NTS1 in the dorsal striatum (Boudin et al., 1996; Ervin et al., 1981; Peltonen et al., 2012). In congruence with these studies, our findings demonstrate that the NAc plays an important role in the locomotor effects of NT, specifically NTS1 activation in the NAc is sufficient to reduce locomotor activity.

In addition, we demonstrated that PD149163 preferentially blocks D2R-mediated hyperactivity compared to that of D1R. Consistent with our findings, NT is known to antagonize the effects of dopamine D2R through NTS1. In the presence of NT, NTS1 reduces the affinity D2R for dopamine by directly interacting with the receptor (Agnati et al., 1983; Koschitzky et al., 2011). NTS1 also inhibits D2R function by promoting D2R internalization and by inhibiting downstream signaling, which may occur without formation of NTS1-D2R heterodimers (Borroto-Escuela et al., 2013; Jomphe et al., 2006; Thibault et

al., 2011). Thus, PD149163 may reduce locomotor activity by inhibiting D2R function. D2R activation is thought to prevent increased striatal medium spiny neuron excitability. It is plausible that NTS1 activation may reduce locomotor activity by increasing the activity of striatopallidal neurons that express D2R. In fact, NT has been shown to block D2R-mediated suppression of accumbal GABAergic neurons (Li et al., 1995).

Our findings suggest that NTS1 agonists may be clinically useful in treating hyperactivity. Increased motor activity is one of the key features of mania in bipolar patients and is thought to be related to overactivation of the dopaminergic system (Minassian et al., 2010). One treatment option for mania is atypical antipsychotics which are considered to partially work by transiently inhibiting D2R as opposed to potentially blocking D2R as observed with typical antipsychotics (Grunder et al., 2003; Perlis et al., 2006). Atypical antipsychotics are associated with similar efficacy and a lower risk of developing extrapyramidal side effects compared to typical antipsychotics (Cipriani et al., 2011). Although the risk of tardive dyskinesia (chronic involuntary movements caused by D2R blocker exposure) is lower with atypical antipsychotics, patients can still develop extrapyramidal side effects (Gao et al., 2008). Therefore, new pharmacological interventions for treating manic hyperactivity that do not directly interact with D2R would be safer and potentially more effective. Interestingly, atypical antipsychotics increase NT expression preferentially in the ventral rather than the dorsal striatum (Merchant and Dorsa, 1993). Because PD149163 is known to exhibit antipsychotic-like effects such as facilitating prepulse inhibition and blocking psychostimulant-mediated hyperactivity (Feifel et al., 2008; Feifel et al., 2010), it could be a candidate intervention for psychomotor agitation associated with psychosis, which is present both in mania and schizophrenia. Here we demonstrate that PD149163 reduces hyperactivity which is likely through activating NTS1 in the NAc. This study further supports the idea for the potential use of NTS1 agonists in the treatment of mania-related behaviors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The NTS1 selective agonist PD149163 reduced basal and novelty-induced activity of C57BL/6J mice.
- NTS1 activation attenuated dopamine receptor-mediated hyperactivity.
- Activation of NTS1 in the nucleus accumbens reduced locomotion, but not in the medial prefrontal cortex.

Fig. 1.

Effect of PD149163 (PD) on locomotor activity of mice in the open-field. **(A)** Total distance traveled in 1 h of mice placed in the open-field 30 min after PD149163 (*i.p.*). PD149163 dose-dependently reduced locomotor activity in the open-field. * $p < 0.05$ relative to saline-treated mice by Tukey tests. # $p < 0.05$ relative to 0.1 mg/kg-treated mice by Tukey test. $n = 8$ for 0.5 mg/kg-treated mice and $n = 9$ for other treatments. **(B)** Data were also analyzed by distance traveled per 10 min in the open-field which revealed the dose-dependent effects of PD149163 over time. Individual comparisons were made by Tukey tests and significance is indicated where $p < 0.05$. Symbols: * 0.1 and 0.5 mg/kg is different from saline, # all doses different from saline, & 0.1 mg/kg different from 0.5 mg/kg, \$0.1 and 0.5 mg/kg different from 0.05 mg/kg. **(C)** Mice recover from the locomotor depressant effect of PD149163 (0.1 mg/kg, *i.p.*) after 3.5 h. Total distance traveled in 15 min was determined every hour. * $p < 0.05$ for PD149163-relative to saline-treated mice by Tukey tests. $n = 6$ for saline- and $n = 7$ for PD149163-treated mice. Data are presented as mean $\pm$ s.e.m.

Fig. 2.

Effect of three or seven repeated PD149163 (0.1 or 0.5 mg/kg, *i.p.*) injections on locomotor activity in the open-field. Mice were placed in the open-field 30 min after the last injection on the test day (day 3 or day 7) and locomotor activity was recorded for 1 h. Acutely treated mice received PD149163 only on the test day and control mice received only saline. **(A)** Mice developed tolerance to the hypolocomotor effect of 0.1 mg/kg of PD149163 after seven repeated administrations. $*p < 0.05$ by Tukey tests. $n = 6$. **(B)** Mice also developed tolerance to the hypolocomotor effect of 0.1 mg/kg of PD149163 after three repeated administrations. $*p < 0.05$ by Tukey tests. $n = 5$ for saline-treated mice and $n = 6$ for other treatments. **(C)** Acute and three days of chronic administration of 0.5 mg/kg of PD149163 similarly reduced locomotor activity in the open-field relative to saline-treated mice. $*p < 0.05$ by Tukey tests. $n = 8$. **(D)** Acute ($n = 6$) and seven days of chronic ($n = 8$) treatment of 0.5 mg/kg of PD149163 similarly reduced locomotor activity in the open-field relative to saline-treated ($n = 8$) mice. $*p < 0.05$ by Tukey tests. Data are presented as mean $\pm$ s.e.m.

Fig. 3.

Effect of PD149163 (PD) on homecage activity of mice. Mice were individually housed with lights on a 6 AM and off at 6 PM. PD149163 (0.5 mg/kg, *i.p.*) or saline was administered at approximately 5 PM, just before the start of the nocturnal active phase, for three days. Baseline (day 0), post-injection (days 1-3) and recovery (day 4) activities were recorded. (A) A representative actogram of the pattern of activity of a mouse treated with saline for three days. Vertical bars represent bouts of activity and bar heights represent activity intensity. Black and white boxes above the actogram indicate dark and light phases, respectively. Diamonds indicate when the injections were administered. (B) A representative actogram of the pattern of activity of a mouse treated with PD149163 for three days. PD149163 given just before lights off, or start of a new nocturnal active phase, decreased homecage activity relative to saline-treated mice. (C-E) PD149163 (0.5 mg/kg, *i.p.*) reduced activity duration on days 1-3 during the early dark phase (18-0 h or 6 PM-12 AM) but not during late dark phase (0-6 h or 12 AM-6 AM) or during the light phase (6-18 h or 6 AM-6 PM). * $p < 0.05$ by Tukey tests. (F-H) PD149163 (0.5 mg/kg, *i.p.*) reduced activity intensity during early dark phase (18-0 h) but not during late dark phase (0-6 h) or during the light phase (6-18 h). * $p < 0.05$ for effect of treatment by two-way repeated measures ANOVA. $n = 5$ for saline- and $n = 7$ for PD149163-treated mice. Data are presented as mean $\pm$ s.e.m.

Fig. 4.

Effect of PD149163 (PD) on rotarod performance and grip strength. **(A)** PD149163 (0.1 and 0.5 mg/kg, *i.p.*) reduced the time mice were able to stay on the rotarod relative to baseline performance. Mice recovered from 0.1 mg/kg of PD149163 after 2.5 h. * $p < 0.05$ relative to time 0 performance (Tukey tests). $n = 8$ for 0.1 mg/kg and $n = 9$ for 0.5 mg/kg-treated mice. **(B)** PD149163 (0.5 mg/kg, *i.p.*) did not affect the grip strength of mice tested 30 min after injection relative to saline-treated mice. $n = 8$. **(C)** PD149163 (0.5 mg/kg, *i.p.*) did not affect the grip strength of mice tested 1 h after injection relative to saline-treated mice. $n = 7$. Data are presented as mean $\pm$ s.e.m.

Fig. 5.

Effect of PD149163 (PD) on dopamine receptor-mediated hyperactivity. **(A)** PD149163 (0.05 or 0.1 mg/kg, *i.p.*) or saline (sal) was administered 15 min before D2R agonist bromocriptine (bromo, 8 mg/kg, *i.p.*) or vehicle (veh) treatment. Mice were placed in the open-field 3 h after the last injection for 1 h. Pretreatment with both doses of PD149163 inhibited the hyperactivity induced 3 h after the bromocriptine injection. $*p < 0.05$ (Tukey test). $n = 8$ for saline + vehicle-, $n = 6$ for saline + bromo-, $n = 7$ for PD 0.05 + bromo- and $n = 8$ for PD 0.1 + bromo-treated mice. **(B)** PD149163 (0.05 or 0.1 mg/kg, *i.p.*) or saline was administered 15 min before D1R agonist SKF81297 (SKF, 8 mg/kg, *i.p.*) or saline. Locomotor activity of mice was recorded in the open-field 15 min after the last injection. Only 0.1 mg/kg of PD149163 reduced SKF81297-induced hyperactivity. $*p < 0.05$ (Tukey test). $n = 7$ for saline + saline-, $n = 6$ for saline + SKF-, $n = 6$ PD 0.05 + SKF- and $n = 5$ PD 0.1 + SKF-treated mice. **(C)** PD149163 (0.05 mg/kg, *i.p.*) or saline was administered 2.5 h after bromocriptine (8 mg/kg, *i.p.*) or vehicle treatment. PD149163 still reduced D2R-mediated hyperactivity when administered after bromocriptine. $*p < 0.05$ (Tukey test). $n = 6$ for saline + vehicle-, $n = 8$ for saline + bromo-, and $n = 7$ for PD 0.05 + bromo-treated mice. Data are presented as mean $\pm$ s.e.m.

Fig. 6.

Effect of microinjection of PD149163 (PD) into the NAc and mPFC on locomotion of mice. **(A)** Observed location of injectors after microinjection into the NAc. **(B)** Total distance traveled per 10 min of mice placed in the open-field 30 min after PD149163 (2 pmol) microinjection into the NAc. Intra-NAc administration of PD149163 reduced locomotor activity relative to saline-microinjected mice. $*p < 0.05$ by Tukey tests. $n = 6$. **(C)** Observed location of injectors after microinjection into the mPFC. **(D)** Total distance traveled per 10 min of mice placed in the open-field 30 min after PD149163 (2 pmol) microinjection into the mPFC. Intra-mPFC administration of PD149163 did not affect the locomotor activity as compared to mice microinjected with saline. $n = 5$ for saline- and $n = 6$ for PD149163-treated mice. Data are presented as mean $\pm$ s.e.m.