Pharmacology, Biochemistry and Behavior 98 (2011) 181–187

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Characterization of the selective mGluR1 antagonist, JNJ16259685, in rodent models of movement and coordination

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ARTICLE INFO

Article history:
Received 22 July 2010
Received in revised form 22 November 2010
Accepted 23 November 2010
Available online 29 November 2010

Keywords:
Metabotropic glutamate receptor
mGluR1
Learning
Motor
Movement
Coordination

ABSTRACT

Metabotropic glutamate receptor 1 (mGluR1) antagonists interfere with learning and memory; however, their role in motor function is not well elucidated despite their abundance in brain areas implicated in the control of movement. Here, the effects of mGluR1 antagonism on movement, coordination, and motor learning were investigated. JNJ16259685, a selective mGluR1 antagonist (negative allosteric modulator), was tested in assays of motor skill, and motor learning in rats and mice. JNJ16259685 produced very minimal effects on locomotor activity and posture up to a dose of 30 mg/kg. Motor skill was unaffected for well-learned tasks (up to 30 mg/kg) in rats, but impaired in mice. Both rats and mice rats were profoundly impaired (0.3 mg/kg) in the acquisition of a novel motor skill (rotarod). These results implicate the mGluR1 receptor in the acquisition of novel motor skills. JNJ16259685 dramatically reduced rearing behavior, exploration of a novel environment and lever pressing for a food reward (rat: 0.3 mg/kg; mouse: 1 mg/kg). JNJ16259685 (30 mg/kg) had no effect on reflexive startle responses to loud auditory stimuli or foot shock in mice. Previous groups have proposed that mGluR1 antagonists induce a general reduction in motivation. The effects seen here to reduce exploration and reward are consistent with that hypothesis. Pharmacological inhibition of the mGluR1 receptor has a modest effect on motor function but blocks motor learning and may reduce motivation to perform simple behaviors.

1. Introduction

Metabotropic glutamate receptors (mGluRs) are a family of eight G-protein coupled receptors categorized into three groups based on sequence homology (Pin and Duvoisin, 1995). Group 1 receptors, which include mGluR1 and mGluR5, are Gq-coupled, excitatory, and primarily post-synaptic (Schoepp, 2001). mGluR1 receptors have been shown to be involved in a wide variety of neurophysiological processes including nociception (Varty et al., 2005; Sasikumar et al., 2009), anxiety (Steckler et al., 2005a; Pietraszek et al., 2005; Mikulecka and Mares, 2009; Klodzinska et al., 2004), ethanol self-administration (Besheer et al., 2008), depression (Belozertseva et al., 2007), epilepsy (Shannon et al., 2005), neuroprotection (Bruno et al., 2001) and Parkinson's disease (Ossowska et al., 2003). These findings have produced an interest in the potential of mGluR1 receptors for the treatment of central nervous system (CNS) related disorders in man (Niswender et al., 2005).

Given the importance of glutamate across numerous critical processes, and the wide distribution of mGluR1 receptors in the CNS, the potential for unwanted side effects from mGluR1 antagonism must be carefully considered. In rodent models of learning and memory, for example, pharmacological blockade of the mGluR1 receptor has been reported to disrupt learning in the Morris water maze (Steckler et al., 2005b; Mikusa et al., 2005;), the passive avoidance assay (Gravius et al., 2005;), as well as auditory and contextual fear conditioning (Gravius et al., 2006; Reidel et al., 2002). The vast majority of studies assessing both beneficial and deleterious effects of mGluR1 inhibition have been conducted in rodent models, which necessitate the use of behavior. However, there is evidence that mGluR1 involvement in movement and coordination, which is an important factor to consider when interpreting the data of a behavioral assay. mGluR1 receptors have been shown to be richly distributed in regions associated with motor function including the cerebellum (Shigemoto et al., 1992; Fotuhi et al., 1993), and basal ganglia (Conn et al., 2005). Moreover, behavioral evidence in rodents suggests a functional role for mGluR1 in motor behaviors. For example, mice lacking the mGluR1 receptor have been reported to have impairments in motor coordination in the rotarod test (Aiba et al., 1994). It has also been demonstrated that synaptic plasticity in

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doi:10.1016/j.pbb.2010.11.018

regions associated with motor function, is impaired by mGluR1 hypofunction. Aiba et al. (1994) reported that knocking out the mGluR1 receptor disrupts cerebellar long-term depression (LTD) while Gubellini and colleagues reported that cortico-striatal long-term potentiation (LTP; Gubellini et al., 2003) and LTD (Gubellini et al., 2001) are dependent on mGluR1 activation. Any effects that mGluR1 antagonists have on rodent motor function complicate the interpretation of findings in other assays.

The present studies were designed to systematically assess the effect of pharmacological inhibition of mGluR1 on motor function and motor learning in rodents. Here, we employed the potent and selective mGluR1 antagonist, JNJ16259685 (Lavreysen et al., 2004a), as a tool to investigate the effects of acute mGluR1 antagonism on motor functioning. JNJ16259685 is selective over mGluR5 as well as group 2 and 3 metabotropic glutamate receptors and is efficacious in rodent models of anxiety (Steckler et al., 2005a).

2. Methods

2.1. Animals

Male C57BL/6J mice (Jackson Labs, Bar Harbor, ME, USA) weighing 25–30 g, and male Long–Evans rats (Charles River, Wilmington, MA, USA) weighing 180–250 g were used in all rat and mouse studies, respectively. The animals were group-housed (mice: 5 per cage; rats: 3 per cage), with the exception of the rats used in the FR-10 study, which were singly housed. Throughout the studies, animals were allowed free access to food (FR-10 rats were food restricted to increase motivation to lever press for a food reward) and water under a 12 h light–dark cycle (lights on 7 a.m.) with constant temperature and humidity. All studies took place during the light cycle between 08:00 and 17:00 h. Animal care and testing procedures were conducted in conformity with the Institutional Animal Care and Use Committee (IACUC), and in compliance with the NIH 'Guide to the Care and Use of Laboratory Animals' and the Animal Welfare Act.

2.2. Drugs

JNJ16259685 [(3,4-dihydro-2H-pyrano[2,3]b quinolin-7-yl) (cis-4-methoxycyclohexyl) methanone] was synthesized by the Medicinal Chemistry department at the Schering-Plough Research Institute. The compound was suspended in 10% hydroxypropyl-betacyclodextrin (HPβCD) and injected subcutaneously (sc) 30 min prior to testing, except where otherwise noted. (+)MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate, Sigma Chemical Co., St. Louis, MO, USA] was administered in 0.9% saline and delivered intraperitoneally (ip) 30 min prior to testing. Dose volumes for the rat and mouse were 2 ml/kg and 10 ml/kg, respectively. Pilot data indicated that exposure in rats and mice peaked 30 min after sc injection. Dose ranges were selected based on brain exposures at doses ranging from 0.3 to 30 mg/kg and 1 to 10 mg/kg in mice and rats, respectively (Table 1). Doses are expressed as free base.

Table 1
Plasma and brain concentrations of JNJ16259685 in mice.

Dose (mg/kg)	Plasma (ng/mL)		Brain (ng/g)		B/P ratio	
	Mouse	Rat	Mouse	Rat	Mouse	Rat
0.3	16 ± 7		33 ± 11		2.09	
1	80 ± 58	720 ± 329	63 ± 11	188 ± 20	0.79	0.26
3	186 ± 92	1322 ± 242	92 ± 9	534 ± 20	0.49	0.40
10	336 ± 241	3782 ± 335	159 ± 59	1196 ± 154	0.47	0.32
30	1290 ± 508		577 ± 209		0.45	

The data represent plasma and brain exposures of JNJ16259685 in mice 30 min following ip administration. Five animals were used per species per dose. B/P ratio = brain:plasma concentration ratio.

2.3. Irwin

This procedure, used to measure overt behavioral, neurological and autonomic responses to the drug challenge, was based on the methods described by Irwin (1964). Briefly, rats were randomly separated into four groups ($n = 6$), each of which received a different dose (0, 3, 10, or 30 mg/kg) of JNJ16259685. An expert observer, blind to the drug treatment of the animals, assessed and scored the animals at 30, 60, 120, and 240 min post-injection. The animals were assessed for passivity, body elevation, limb position, limb tone, body tone, gait, and pupil size. For each of these behaviors, a score of 0 was assigned to animals that appeared "normal", whereas scores of ±1, ±2, or ±3 indicated mild, moderate, or severe increases (+) or decreases (−) from normality. Individual animals that received a score of ±2, or greater, were considered to be significantly effected on the measure. A dose was considered to have a significant effect if 3 or more of the animals received a score of greater than ±2. Table 2 represents the number of animals in each treatment group that were scored as significantly impaired on each behavioral measure.

2.4. Locomotor activity

Rats and mice were transferred in their home cages from the colony room to the testing area and allowed to habituate in an anteroom for at least 60 min before being randomly separated into dose groups (rat doses: 0, 0.1, 0.3, 1, 3, or 10 mg/kg; mouse doses: 0, 1, 3, or 10 mg/kg; $n = 10$ per group). Thirty min following injection, activity levels were recorded for 1 h. The rat study used a Versamax LMA system (Accuscan Instruments, Columbus, OH). Each monitoring system consisted of a Plexiglas box (height: 30 cm; width: 42 cm; length: 42 cm) and horizontal activity was monitored by *XY* axis photobeams (sampling rate: 100 Hz) located 2 cm above the floor and spaced 2.5 cm apart. Photobeams located 20 cm from the floor recorded the number of rears. The center of the chamber was defined by the inner square measuring 20 × 20 cm (400 cm^2) and the total distance traveled in this center area was recorded separately from total overall distance traveled, but was included in the total distance measure. The mice were individually placed in a Plexiglas chamber (24 × 24 cm) and allowed to explore for 1 h. During this time, photo beams mounted on the chamber walls (Coulbourn Instruments, Allentown, PA, USA) measured total distance traveled, center distance (center of the chamber measured 20 × 20 cm) and the number of rears. Beam break counts were transferred to a computer that digitized and stored the data for subsequent analysis. Data were stored in 5 min bins.

2.5. Rat rotarod

For both rats and mice, rotarod testing took place over two consecutive days. On day 1, the animals were trained until they were able to remain on the rod (speed = 16 RPM) for 120 s for two trials. The animals were given as many trials as required to reach this performance criterion, after which they were returned to their home cage until testing the following day. Animals that did not reach this criterion were eliminated from the study (<5%). On the test day, the animals were randomly assigned to a dose group (rat: 0, 0.3, 1, 3, 10, or 30 mg/kg; $n = 9$; mouse: 0, 1, 3, or 10 mg/kg, $n = 9$) and tested for three trials on the rod rotating at 16 RPM. The animals were allowed a minimum 30 s rest period between trials. Scores on the three trials were averaged for each animal. The dimensions of the rat rotarod were 8.5 cm width and 7 cm diameter. The dimensions of the mouse rotarod were 3 cm width and 3 cm diameter.

In order to assess the effect of JNJ16259685 on motor skill acquisition, naïve animals were transferred to the procedure room,

Table 2
Results of rat Irwin observations after treatment with JNJ16259685.

Dose:	Veh				3 mg/kg				10 mg/kg				Brain (ng/g) B/P ratio			
Time (min):	30	60	120	240	30	60	120	240	30	60	120	240	30	60	120	240
Passivity	0	0	0	0	0	0	0	0	2	2	0	0	2	2	1	4
Body elevation	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	0
Limb position	0	0	0	0	0	0	0	0	2	2	0	0	1	1	1	0
Limb tone	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	0
Body tone	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Change in gait	0	0	0	0	0	0	0	0	2	2	0	0	4	3	3	1
Pupil size	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Behavioral effects of JNJ16259685 in the rat 30, 60, 120, and 240 min following injection. The values represent the number of animals out of six who received scores of greater than or equal to 2, or less than or equal to −2 for each measure. Six rats were tested at each dose.

randomly assigned to dose groups ($n=10$), and injected with the appropriate dose of JNJ16259685. Thirty min later, the animals were placed on the rotating rod (speed = 8 RPM) and the latency to fall from the rod was recorded by an observer blind to the treatment of the animals. Testing on the rotarod was repeated for eight trials for the rats and ten trials for the mice with a minimum 30 s rest period between trials. For all studies, the maximum time for an individual trial was 120 s.

2.6. Beam walking

Twenty-four h prior to testing, rats were pre-trained to successfully traverse a beam (length: 90 cm; width: 2 cm; elevation: 43 cm) within 60 s without any foot slips on two successive trials. On test day, the rats were randomly separated into dose groups (0, 0.3, 1, 3, 10, or 30 mg/kg, $n=9$) and given two trials on the beam. A seven-point scale was developed to measure performance in the rats ranging from falling off the beam immediately (1) to successfully traversing the beam with no slips (7). Additionally, the distance traveled on the beam before the end of the 60 s trial, or before the rats fell from the beam was recorded. All measures were recorded by an expert observer blind to the drug treatment of the animals.

2.7. Fixed-ratio responding

Studies were conducted in operant conditioning boxes housed in sound-attenuating chambers (MED Associates, Georgia, VT). Rats were trained to lever press for 45 mg food pellets (Bio-Serv, Frenchtown, NJ) on a FR-10 schedule of reinforcement. To maintain motivation, rats were kept at approximately 80% of their normal free-feeding body weight. Sessions lasted either 60 min or until a rat had received 100 pellets, whichever occurred first. Custom-designed software controlled the boxes, ran the FR program and collected the data (KESTREL system, Conclusive Solutions, Cambridge, UK). All studies were conducted using a within-subjects design in which the animals received every dose of JNJ16259685 (0.03, 0.3, 3, and 30 mg/kg, $n=12$) with a minimum two-day washout period between tests.

2.8. Response to auditory stimuli and foot shock

Mice were randomly divided into either vehicle or JNJ16259685 (30 mg/kg, $n=10$) groups. Mice were acclimated to the startle chambers for 60 s with a 65 dB background level of noise. Auditory stimuli (stimulus intensities: 65, 72, 81, 90, 98, 106, 114, and 121 dB; stimulus duration: 40 ms) were delivered 6 times each in random order to the animals with a random interval which ranged from 15 to 25 s. Startle responses were recorded for 65 ms beginning at the onset of the auditory stimulus. Startle responses across the 6 exposures were averaged for each stimulus.

Five min following the startle procedure, the mice were placed on a seven-rod shock grid. Following 3 min of acclimation, foot shocks (intensities: 0, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 mA; duration: 1 s) were delivered to the animal 21 times (3 times per intensity) in a random order and at variable intervals ranging from 30 to 40 s. The magnitude of response to the foot shock was recorded for 1 s beginning at the onset of the delivery of shock. Startle responses were recorded automatically and averaged across the three exposures for each shock intensity.

2.9. Statistical analysis

A one-way repeated-measures ANOVA was used to analyze the FR-10 data. Two-way ANOVAs were used to analyze the rat motor learning (dose × trial), and the mouse auditory and foot shock startle (dose × intensity) data. For the rat beam-walk test a Kruskal Wallis test was used to analyze the nonparametric skill measure. Data from the remaining studies were analyzed using one-way ANOVAs comparing the dose groups. A Dunnett's test was used as a post-hoc measure for all significant tests. Significance was defined as $p<0.05$.

3. Results

3.1. Rat Irwin

Rats treated with either vehicle or 3 mg/kg JNJ16259685 displayed no overt behavioral effects (Table 2). A 10 mg/kg dose of JNJ16259685 produced moderate effects on passivity, limb position, and gait at 30 and 60 min, but no effects were seen at 120 or 240 min. A dose of 30 mg/kg of JNJ16259685 significantly affected passivity, body elevation, and gait and had a moderate effect on limb tone. All these effects lasted at least 120 min following the delivery of the compound. The increase in passivity was likely a result of a general sedative effect of the compound. Higher doses of JNJ16259685 produced a crouching posture and a 'waddle' when the animals walked, which accounted for the significant scores in body elevation and gait respectively. The 10 mg/kg effect on limb position was a splaying of the limbs.

3.2. Locomotor activity

Administration of JNJ16259685 to rats produced a significant main effect on locomotor activity [$F(5,54)=3.78$, $p<0.01$] with doses of 0.3–10 mg/kg all reducing total distance relative to vehicle (Fig. 1A). Center distance was significantly reduced [$F(5,54)=2.5$, $p<0.05$] with doses of 0.3–3 mg/kg significantly reducing center time relative to vehicle (Fig. 1B). Distance traveled in the periphery was also reduced [$F(5,54)=3.23$, $p<0.05$], but only the 3 and 10 mg/kg doses were significantly lower than vehicle (data not shown). In addition, the total number of rears the rats made during the 60 min session was significantly reduced at all doses compared to vehicle [$F(5,54)=15.35$, $p<0.01$] (Fig. 1C).

There was no significant effect of JNJ16259685 on of total distance traveled in mice [$F(3,35)=1.82$, $p>0.05$] (Fig. 1D). However, both center distance [$F(3,35)=7.21$, $p<0.01$] and the number of rears [$F(3,35)=29.91$, $p<0.01$] were significantly reduced. All three drug-treated groups

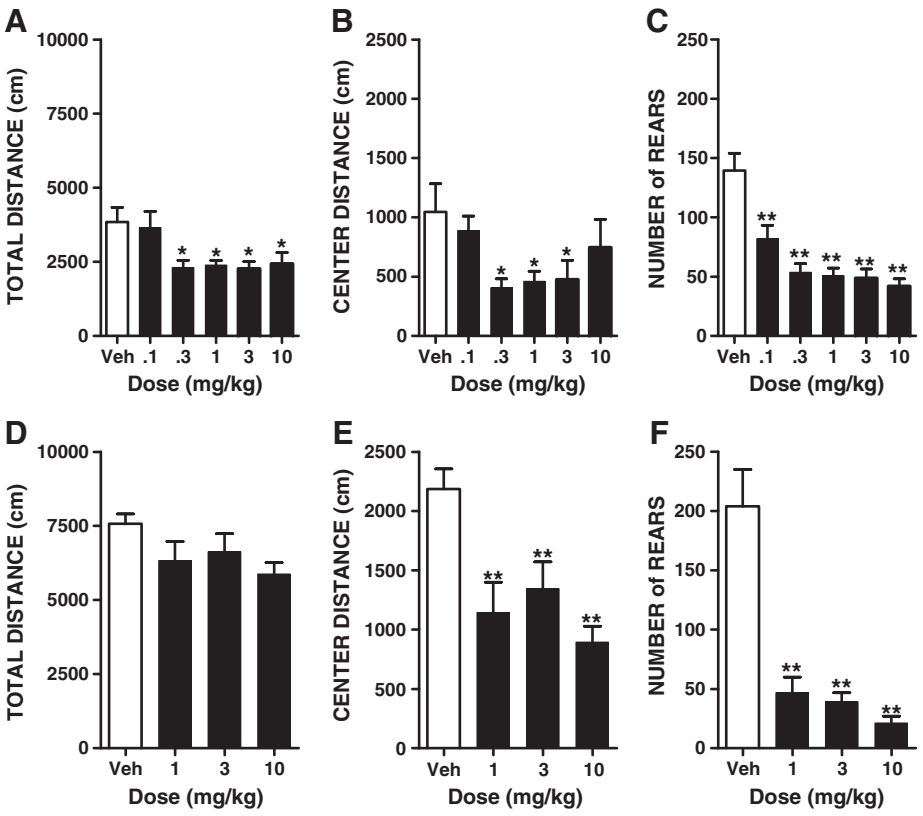

Fig. 1. The effects of JNJ16259685 on activity levels in the rat and mouse. Rats displayed a reduction in total distance (A) at doses of 0.3–10 mg/kg. The majority of this reduction is attributable to a drop in center distance (B). JNJ16259685 induced a robust decrease in the number of rears made by the animals at all doses (C). In the mice, baseline locomotor activity was not affected by drug treatment (D), however there was a significant drop in center distance (E). Like the rat, the mice displayed a robust decrease in rearing behavior in response to JNJ16259685 (F). Bars represent mean ± SEM, $n = 9–10$ per group. $^{*}p<0.05$; $^{**}p<0.01$ vs. vehicle (Veh).

(1, 3, and 10 mg/kg) were significantly lower than vehicle on both of these measures (Fig. 1E and F).

In both rodent species, center distance, as a percent of total distance traveled was also reduced by drug treatment. In vehicle-treated rats, center distance made up 30% of the total distance traveled. This percentage was reduced to 26%, 17%, 18%, 18% and 28% at doses of 0.1, 0.3, 1, 3, and 10 mg/kg, respectively. JNJ16259685 had a similar effect in the mice. Center distance in vehicle-treated mice made 30% of their total distance traveled. This was reduced to 18%, 21%, and 15% in mice treated with 1, 3 and 10 mg/kg, respectively.

3.3. Rotarod

When administered to pre-trained rats, JNJ16259685 had no effect on rat rotarod performance [$F(5,48) = 0.64$, $p>0.05$] (Fig. 2A). However, there was a main effect of JNJ16259685 [$F(4,41) = 7.22$, $p<0.01$] on mouse rotarod performance, and all four dose groups had shorter latencies to fall from the rod relative to the vehicle group (Fig. 2B).

In the motor-learning procedure, there was a significant effect of dose in the rat [$F(3,36) = 15.45$, $p<0.01$] and the mouse [$F(4,45) = 43.92$, $p<0.01$]. JNJ16259685 significantly decreased latency to fall across trials in both rats (Fig. 3A) and mice (Fig. 3B). A significant dose by trial interaction in rats [$F(21,252) = 4.73$, $p<0.01$] and mice [$F(36,405) = 4.95$, $p<0.01$] indicated that there was a significant difference in the rate of learning of the drug groups relative to vehicle.

3.4. Beam walking

There was a statistically significant effect of JNJ16259685 on performance on the beam-walking test but only the 1 mg/kg group was statistically impaired relative to vehicle (Fig. 4). There were no observable impairments on the behavior of the animals. All of the animals tested in all dose groups were able to traverse the full 90 cm length of the beam without falling.

3.5. Fixed-ratio responding

JNJ16259685 dose-dependently reduced lever pressing for a food reward [$F(4,55) = 26.97$, $p<0.01$]. Performance of the animals was

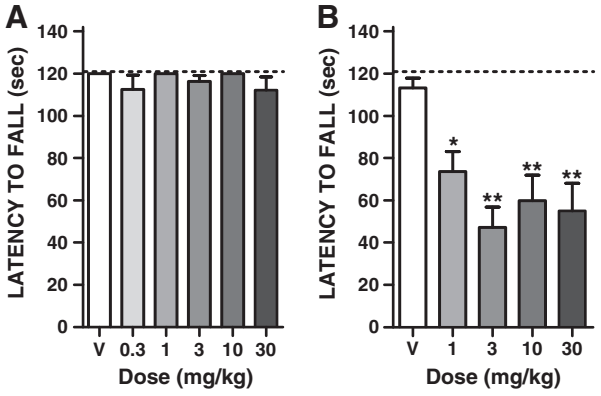

Fig. 2. The effects of JNJ16259685 on motor coordination in the rat and mouse. In the rat rotarod test (A), JNJ16259685 did not impair performance on previously trained animals up to a dose of 30 mg/kg. However in the mouse, JNJ16259685 (1–30 mg/kg) did impair performance on the rotarod (B). Bars represent mean ± SEM, $n = 9–10$ per group. $^{*}p<0.05$; $^{**}p<0.01$ vs. vehicle (V).

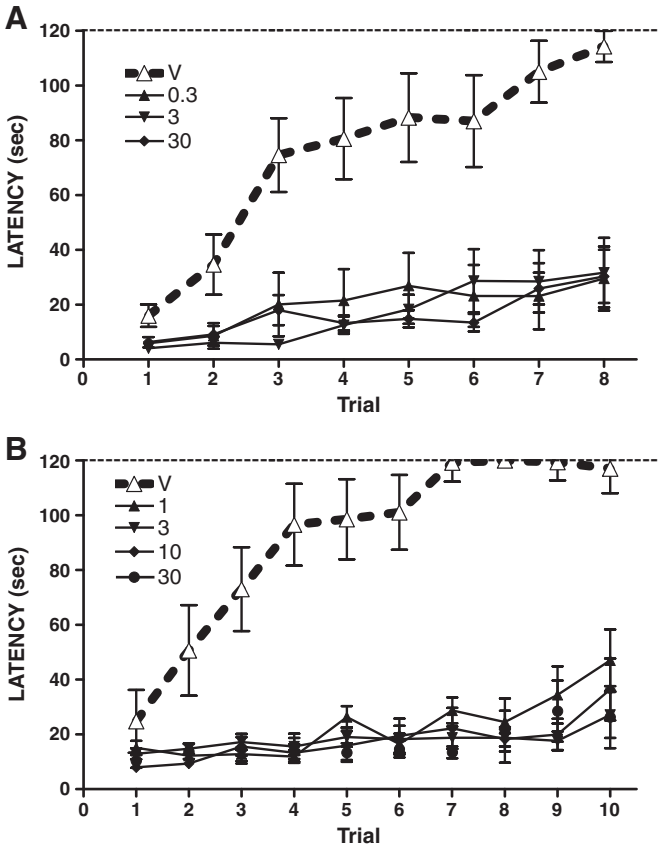

Fig. 3. The effect of JNJ16259685 on motor skill acquisition in rats and mice. In naïve animals, JNJ significantly impaired acquisition of a motor skill (rotarod) in rats (A) and mice (B). In rats, 9 of the 10 animals in the vehicle group remained on the rotarod for 120 s, whereas only two of the JNJ16259685-treated animals (1 at 0.3 mg/kg; 1 at 3 mg/kg) were able to remain on the rotarod for 120 s on trial 10. Similarly, 9 of 10 vehicle-treated mice remained on the rotarod on trial 10, whereas none of the mice that received any dose of JNJ16259685 was able to remain on the rod. The number of trials was truncated at 8 and 10 for the mouse and rat, respectively. Bars represent mean ± SEM, $n = 10$ per group.

significantly impaired relative to vehicle treatment at doses from 0.3 to 30 mg/kg (Fig. 5).

Animals treated with doses of 0.03, 0.3, 3, and 30 mg/kg suppressed responding by 18%, 48%, 77% and 80%, respectively relative to vehicle-treated animals.

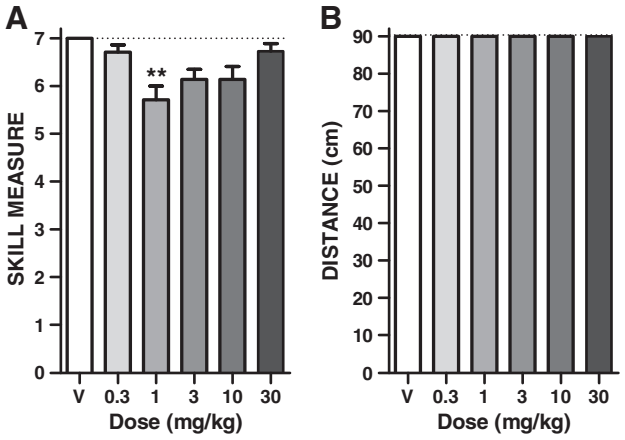

Fig. 4. Effect of JNJ16259685 on rat beam-walking performance. The compound did slightly impair the coordination of the animals to cross a narrow beam (A) at a dose of 1 mg/kg, but not at higher doses. All of the animals tested were able traverse the full 90 cm beam without falling off or stopping (B). Bars represent mean ± SEM, $n = 9$ per group. $^{**}p < 0.01$ vs. vehicle (V).

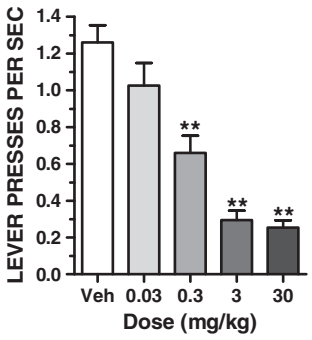

Fig. 5. The effects of JNJ16259685 on lever pressing under a fixed-ratio FR-10 schedule of reinforcement. The average number of lever presses per second was reduced by 19%, 48%, 77%, and 80% respectively by treatments with 0.03, 0.3, 3, and 30 mg/kg of JNJ16259685. $^{**}p < 0.01$ vs. vehicle (Veh).

3.6. Response to auditory stimuli and foot shock

There was no significant main effect of JNJ16259685, nor a significant drug by intensity interaction in the mouse auditory startle (Fig. 6A) and foot shock startle (Fig. 6B) paradigms.

4. Discussion

Evidence from knockout mice suggests that the mGluR1 receptor plays a role in motor function (Aiba et al., 1994). This is consistent with the distribution of mGluR1 receptors in regions known to be involved in motor function such as the cerebellum and basal ganglia (Lavreysen et al., 2004b). Less is known about the potential motor impairments that might result from a pharmacologically-induced

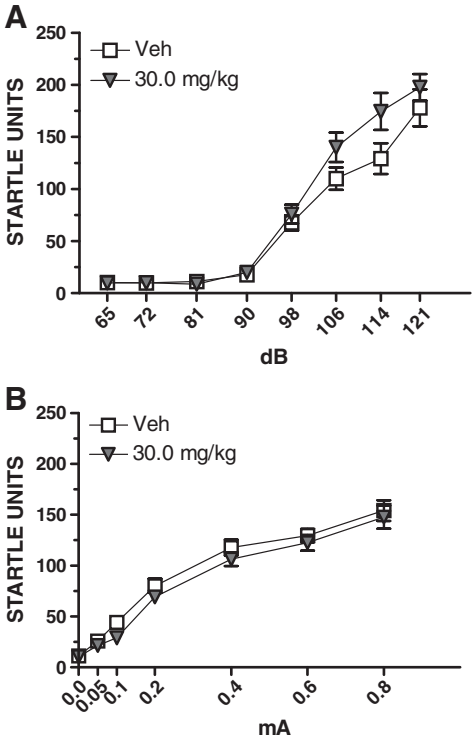

Fig. 6. The effects of JNJ16259685 on startle response to auditory stimuli and foot shock. Mice pretreated with 30 mg/kg of JNJ16259685 showed no change in responsiveness to an auditory stimulus (A) or a foot shock (B) as measured by the startle response. Bars represent mean ± SEM, $n = 9$ per group.

blockade of mGluR1 activity. In the present studies, we investigated the effects on rodent behavior of JNJ16259685. In rats, treatment with a 30 mg/kg dose of JNJ16259685, and with 10 mg/kg to a lesser extent, produced obvious effects when the animals in the Irwin assay. The effects are consistent with the interpretation that the compound negatively impacts movement and coordination. Further testing was done in an attempt to quantify these motor assays.

In rats, there was a very mild effect on motor function and coordination on the rotarod and beam-walking tests. This finding is consistent with the findings of Varty et al. (2005) who reported only very modest effects on rat motor function following treatment with the mGluR1 antagonist, LY456236. Similarly, Kohara et al. (2005) reported very mild effects of the selective mGluR1 receptor antagonist YM-298198 on rat motor performance.

The motor coordination data in the mouse contrast with the rat findings and are at odds with published findings from Kohara et al. (2005) who reported that pharmacological inhibition of the mGluR1 receptor has no effect in a one-trial rotarod test in pre-trained mice. Relative to the rat, JNJ16259685 had a much more profound effect on mouse motor performance producing a significant reduction in the time on the rod at a dose as low as 1 mg/kg. El-Kouhen et al. (2006) reported that the mGluR1 antagonist A-841720 reduced spontaneous locomotor activity and impaired performance on the rotarod test. It is not clear why there is a difference between the rat and mouse sensitivity. This is clearly not an exposure-driven phenomenon as our pharmacokinetic data demonstrated that rats have brain exposure in the neighborhood of 3–7 times that of the mouse, although the extracellular drug concentration proximal to the receptor was not measured. Steckler et al. (2005a) reported that there was an effect of JNJ16259685 at doses of 0.63 and 1 mg/kg in a rat anxiety model. Unfortunately there are no published anxiety data in the mouse of which we are aware, that allow for a direct comparison of species sensitivity differences in efficacy models. Interestingly, Kazdoba et al. (2006) reported that JNJ16259685 impaired performance in cognition tasks at lower doses in the rat than the mouse, which is consistent with the relative brain exposure. The involvement of mGluR1 receptors in motor skill acquisition is consistent with previously published findings. There is a small, but consistent literature, demonstrating that mGluR1 antagonists impair non-motor learning. Steckler et al. (2005b) reported that JNJ16259685 impaired learning in a Morris water maze, and Gravius et al. (2005) reported that the mGluR1 antagonist, ECQMCM, which is structurally similar to JNJ16259685, disrupted learning in a rat passive avoidance test of memory. Kazdoba et al. (2006) reported that JNJ16259685 disrupted memory across a variety of assays including the Morris water maze, the passive avoidance test, the Y-maze test and fear conditioning. As well, El Kahoun et al. (2006) reported that A-841720 disrupted learning in a Y-maze test of memory.

Also consistent with our results on motor learning, Aiba et al. (1994) reported that mGluR1 knockout mice are impaired on the acquisition of a motor skill and have impaired LTD in the cerebellum. Gubellini et al. (2003) demonstrated that knocking out the mGluR1 receptor impairs LTP in the cortico-striatal pathway. Given the LTP and LTD represent forms of synaptic plasticity with a very well-established link to learning and memory, and given that these effects are found in regions of the brain known to be critical for motor function, the effect of JNJ16259685 on motor learning is predicted by these previously reported findings. To our knowledge, the present finding is the first to demonstrate the effect of acute pharmacological mGluR1 disruption on motor skill acquisition.

JNJ16259685 had a minimal effect on baseline activity levels in both species, as evidenced by the overall effect on locomotor activity in both rats and mice. This effect was consistent with a previously published report by Steckler et al. (2005a) who demonstrated that a dose of 5 mg/kg of JNJ16259685 reduced overall activity in rats. The lack of effect on overall activity levels suggests that the compound induces only a very mild sedative-like effect in the animals. This is consistent with the Irwin measures in the rat—there was a slight effect on limb tone, but no overall effect on body tone.

There was a robust decrease in the amount of time the animals spent in the center portion of the activity chambers, and in the frequency with which the animals reared. Steckler et al. (2005a) did not report data on the effect of the drug on either center distance or rearing behavior; however, Dravolina et al. (2006) reported that EMQMCM reduced rearing in rats. Willingness to enter the center of an open area has been used as a measure of anxiolysis, so a possible explanation of our finding is that JNJ16259685 is anxiogenic. However, this is unlikely given previous reports that suggest that mGluR1 antagonism has anxiolytic-like properties. Steckler et al. (2005a) reported that JNJ16259685 increased licking in a punished-licking assay. Additionally, Varty et al. (2005) reported that the mGluR1 antagonist, LY456236, increased licking in the Vogel Conflict and Conditioned Lick Suppression tests, which are predictive of anxiolysis. These findings indicate that treatment with JNJ16259685 lowers anxiety levels. The lack of center distance could be the result of an increased thigmotaxis. However, this is also an unlikely explanation. In a Morris water maze test, mice dosed with JNJ16259685 demonstrated no thigmotaxis (Kazdoba et al., 2006). Moreover, in our studies, there was no increase; in fact there was a slight decrease, in distance traveled around the perimeter of the activity chambers.

Another potential explanation of the reduction in center distance and rearing is that mGluR1 is involved in general motivation. That is, drug-treated animals experienced a lack of motivation to explore their environment. In the FR-10 task, rats showed reduced lever pressing, which could also be attributed to a reduction in motivation to engage in a behavior for a food reward. Although mGluR1s are located in areas involving the motor processes involved with eating (Sharifullina et al., 2004), the reduction in lever pressing because our animals consumed all food pellets that they pressed for and had no apparent lack of desire to eat when returned to their home cage and given access to food pellets. Behavioral paradigms that more directly assess motivation will be necessary to confirm this hypothesis.

Kazdoba et al. (2006) found that JNJ16259685-treated rats engaging in a Delayed Match-to-Position test of memory, or a Five-Choice test of attention, performed the task as well as vehicle controls, but that there was a dramatic and dose-dependent effect on the extent to which the animals engaged the tasks. These animals, thus, demonstrated no cognitive impairment but also appeared to be less motivated to perform a response-based task. Besheer et al. (2008) reported that JNJ16259685 lowered the motivation to self-administer alcohol. Collectively, these findings suggest that depressing mGluR1 activity decreases general levels of motivation. In opposition to this interpretation, Belozertseva et al. (2007) reported that EMQMCM increased climbing and diving behavior and decreased floating behavior in a rat forced swim test of depression. This latter finding is likely due to anti-depressant effects of the compound as opposed to an effect on motivation levels.

In conclusion, we found that there are relatively mild effects on motor behavior following treatment with JNJ16259685, however the effects are more profound in mouse than rat. Moreover, we tested at doses much higher than required to attain efficacy in models of anxiety (MED: 2.5 mg/kg; Steckler et al., 2005a). This leaves open the possibility that there is room for a small therapeutic window between efficacy and motor side effects. However, the fact that the compound behaves inconsistently across both efficacy models and models of coordination makes its therapeutic window impossible to accurately assess. More troubling was the abolishment of motor learning in rats, which was evident at a dose lower than that required to attain efficacy in animal models of anxiety. Our findings clearly indicate that the effects on rodent behavior are nuanced and depend on the species, the task and prior training. Moreover, these effects need to be considered in the interpretation of results in any model of rodent behavior.

References

Aiba A, Kano M, Chen C, Stanton ME, Fox GD, Herrup K, et al. Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. Cell 1994;79: 377–88.

Belozertseva IV, Kos T, Popik P, Danysz W, Bespalov AY. Antidepressant-like effects of mGluR1 and mGluR5 antagonists in the rat forced swim and the mouse tail suspension tests. Eur Neuropsychopharmacol 2007;17:172–9.

Besheer J, Faccidomo S, Grondin JJ, Hodge CW. Effects of mGlu1-receptor blockade on ethanol self-administration in inbred alcohol-preferring rats. Alcohol 2008;42: 13–20.

Bruno V, Battaglia G, Copani A, Cespedes VM, Galindo MF, Cena V, et al. An activity-dependent switch from facilitation to inhibition in the control of excitotoxicity by group 1 metabotropic glutamate receptors. Eur J Neurosci 2001;13:1469–78.

Conn JP, Battaglia G, Marino MJ, Nocoletti F. Metabotropic glutamate receptors in the basal ganglia motor circuit. Nat Rev Neurosci 2005;6:787–98.

Dravolina OA, Danysz W, Bespalov AY. Effects of group I metabotropic glutamate receptor antagonists on the behavioral sensitization to motor effects of cocaine in rats. Psychopharmacology 2006;187:397–404.

El-Kouhen O, Lehto SG, Pan JB, Chang R, Baker SJ, Zhong C, et al. Blockade of mGluR1 receptor results in analgesia and disruption of motor and cognitive performances: effects of A-841720, a novel non-competitive mGluR1 receptor antagonist. Br J Pharmacol 2006;149:761–74.

Fotuhi M, Sharp AH, Glatt CE, Hwang PM, von Krosigk M, Snyder SH, et al. Differential localization of the phosphoinositide-linked glutamate receptor (mGluR1) and the inositol 1, 4, 5-trisphosphate receptor in the rat brain. J Neurosci 1993;13:2001–12.

Gravius A, Pietreaszek M, Schafer D, Schmidt WJ, Danysz W. Effects of mGluR1 and mGluR5 receptor antagonists on negatively reinforced learning. Behav Pharmacol 2005;16:113–21.

Gravius A, Barberi C, Schafer D, Schmidt WJ, Danysz W. The role of group I metabotropic glutamate receptors in acquisition and expression of contextual and auditory fear conditioning in rats — a comparison. Neuropharmacology 2006;51:1146–55.

Gubellini P, Saulle E, Centonze D, Bonsi P, Pisani A, Bernardi G, et al. Selective involvement of mGlu1 receptors in corticostriatal LTD. Neuropharmacology 2001;40:839–46.

Gubellini P, Saulle E, Centonze D, Costa C, Tropepi D, Bernardi G, et al. Corticostriatal LTP requires combined mGluR1 and mGluR5 activation. Neuropharmacology 2003;44: 8-16.

Irwin S. Drug screening and evaluation of new compounds in animals. In: Nodine JH, Seigler PE, editors. In animal and clinical pharmacologic techniques in drug evaluation. Chicago: Yearbook Medical Publishers, Inc; 2006. p. 55–86.

Kazdoba TM, Leach PT, Lu SX, Bleickardt CJ, Guthrie DH, Parker EM, et al. The mGluR1 antagonist JNJ16259685 impairs performance in select rat and mouse models of cognition. . Program No. 331.7. Abstract Viewer/Itinerary Planner.Atlanta, GA: Society for Neuroscience; 2006. Online.

Klodzinska A, Tatarczynska E, Stachowicz K, Chojnacka-Wojic E, The anxiolytic-like activity of AIDA (1-aminoindan-1,5-dicarboxylic acid), an mGlu1 receptor antagonist. J Physiol Pharmacol 2004;55:113–26.

Kohara A, Toya T, Tamura S, Watabiki T, Yukinori N, Shitaka Y, et al. Radioligand binding properties and pharmacological characterization of 6-amino-N-cyclohexyl-N, 3-dimethylthiazolo[3, 2-a]benzimidazole-2-carboxamide (YM-298198), a high affinity, selective, and noncompetitive antagonist of metabotropic glutamate receptor type 1. J Pharmacol Exp Ther 2005;315:163–9.

Lavreysen H, Wouters R, Bishoff F, Pereira SN, Langlois X, Blokland S, et al. JNJ16259685, a highly potent, selective and systemically active mGlu1 receptor antagonist. Neuropsychopharmacology 2004a;47:961–72.

Lavreysen H, Pereira SN, Leysen JE, Langlois X, Lesage ASJ. Metabotropic glutamate 1 receptor distribution and occupancy in the rat brain: a quantitative autoradiographic study using [3H]R214127. Neuropharmacology 2004b;46:609–19.

Mikulecka A, Mares P. Effects of mGluR5 and mGluR1 antagonists on anxiety-like behavior and learning in developing rats. Behav Brain Res 2009;204:133–9.

Mikusa JP, Cronin EA, Hollingsworth PR, Lehto SG, Zheng G, Stewart AP, et al. The cognitive and locomotor side-effect profile of A-841720, a novel, potent, and selective mGluR1 receptor antagonist in rats. 2005 abstract viewer/itinerary planner. Washington, DC: Society for Neuroscience; 2005. Online.

Niswender CM, Jones CK, Conn PJ. New therapeutic frontiers for metabotropic glutamate receptors. Curr Top Med Chem 2005;5:847–57.

Ossowska K, Wardas J, Pietraszek M, Konieczny J, Wolfarth S. The striopallidal pathway is involved in antiparkinsonian-like effects of the blockade of group I metabotropic glutamate receptors in rats. Neurosci Lett 2003;342:21–4.

Pietraszek M, Sukhanov I, Maciejak P, Szyndler J, Gravius A, Wislowska A, et al. Anxiolytic-like effects of mGlu1 and mGlu5 receptor antagonists in rats. Eur J Pharmacol 2005;514:25–34.

Pin JP, Duvoisin R. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 1995;34:1-26.

Reidel G, Sandager-Nielsen K, Macphail EM. Impairment contextual fear conditioning rats by group 1 mGluRs reversal by nootropic nefiracetam 2002;73:391–9.

Sasikumar TK, Qiang L, Burnett DA, Greenlee WJ, Li C, Heimark L, et al. Tricyclic thienopyridine-pyrimidones/thienopyrimidine-pyrimidones as orally efficacious mGluR1 antagonists for neuropathic pain. Bioorg Med Chem Lett 2009;19:3199–203.

Schoepp DD. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. Pharmacol Exp Ther 2001;299:12–20.

Shannon HE, Peters SC, Kingston AE. Anticonvulsant effects of LY456236, a selective mGluR1 receptor antagonist. Neuropsychopharmacology 2005;49:188–95.

Sharifullina E, Ostroumov K, Nistri A. Activation of group I metabotropic glutamate receptors enhances efficacy of glutamatergic inputs to neonatal rat hypoglossal motoneurons in vitro. Eur J Neurosci 2004;20:1245–54.

Shigemoto R, Nakanishi S, Mizuno N. Distribution of the mRNA for a metobotropic glutamate receptor (mGluR1) in the central nervous system: an in situ hybridization study in the adult and developing rat. J Comp Neurol 1992;322:121–35.

Steckler T, Lavreysen H, Oliveira AM, Aerts N, Van Craenendonck H, Prickaerts J, et al. Effects of mGluR1 receptor blockade on anxiety-related behaviour in the rat lick suppression test. Psychopharmacology 2005a;179:198–206.

Steckler T, Oliveria AFM, Van Dyck C, Van Craenendonck H, Mateus AMA, Langlois X, et al. Metabotropic glutamate receptor 1 blockade impairs acquisition and retention in a spatial water maze task. Behav Brain Res 2005b;164:52–60.

Varty GB, Grilli M, Forlani A, Fredduzzi S, Grzelak ME, Guthrie DH, et al. The antinociceptive and anxiolytic-like effects of the metabotropic glutamate receptor 5 (mGluR5) antagonists, MPEP and MTEP, and the mGluR1 antagonist, LY456236, in rodents: a comparison of efficacy and side-effect profiles. Psychopharmacology 2005;179: 207–17.