Abstract

Rationale: It was recently demonstrated that the priming stimulation effect (PSE) in the runway model of intracranial self-stimulation (ICSS) can be used as a model system to study the motivational effects of drugs. However, the characteristics of this novel experimental model have not been fully clarified.

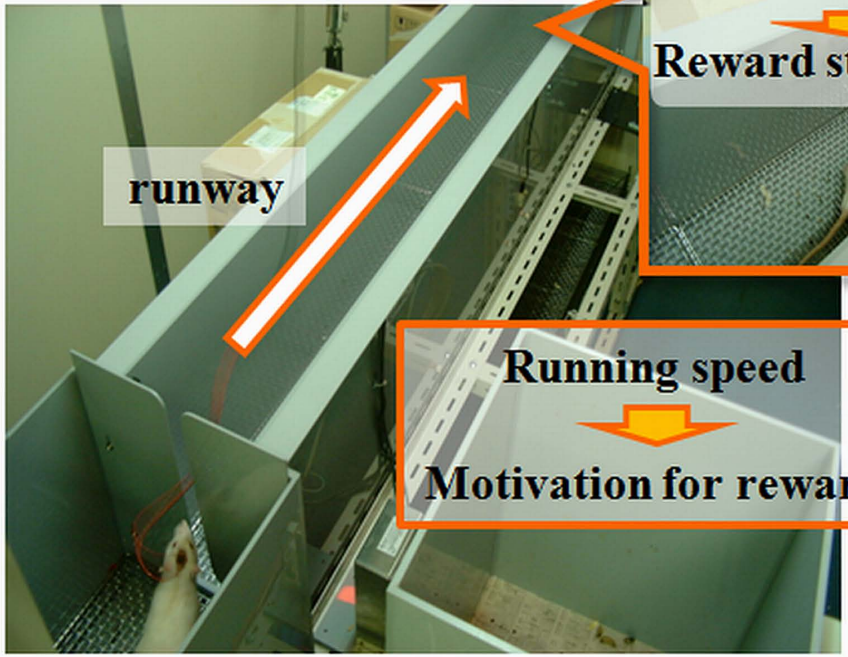
Objective: To elucidate the involvement of dopamine uptake inhibition in motivated behavior and the difference in experimental characteristics between closely related experimental models, we investigated the effects of the dopamine uptake inhibitor GBR12909 in the runway ICSS model, in the forced swimming test (FST), and on conditioned place preference (CPP). In addition, the role of dopamine receptor signaling in the runway model was evaluated using dopamine receptor agonists and antagonists.

Results: GBR12909 dose-dependently increased running speed on the runway and decreased immobility time in the FST without affecting the time spent in the drug-associated compartment in CPP tests. The effect of GBR12909 in the runway model was inhibited by pre-treatment with the dopamine receptor antagonists haloperidol and raclopride. The dopamine receptor agonists SKF38393 and quinpirole dose-dependently decreased running speed.

Conclusions: These results demonstrate that GBR12909 displays motivation-enhancing and antidepressant-like effects without place conditioning effects. In addition, the mechanisms of PSE enhancement in the runway ICSS model are different from those underlying closely associated experimental models and are mediated by increases in dopamine signaling.

Key words: motivation, intracranial self-stimulation, forced swimming test, conditioned place preference, GBR12909, quinpirole

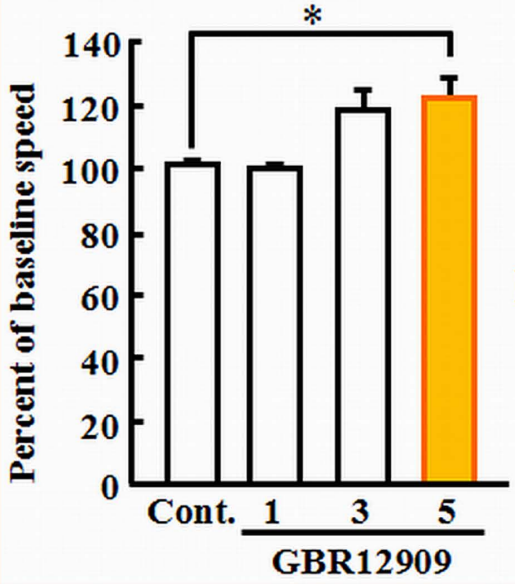
ICSS runway apparatus



Running speed

Motivation for reward

Motivational effect of dopamine uptake inhibitor on the ICSS runway model.



Motivational Enhancement

Highlights

- GBR12909 increased running speed in the runway model of ICSS.
- SKF38393 and quinpirole decreased running speed in the runway model of ICSS.
- Imipramine decreased running speed in the runway model of ICSS.
- Imipramine and GBR12909 decreased immobility time in the forced swimming test.
- GBR12909 did not produce significant place conditioning behavior.

Behavioural Brain Research
Research report

Effect of GBR12909 on affective behavior: Distinguishing motivational behavior from antidepressant-like and addiction-like behavior using the runway model of intracranial self-stimulation.

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1. Introduction

Recent studies reported that certain dopaminergic agents display antidepressant activity in patients with Parkinsonism as well as in patients that are resistant to SSRI treatment [1]. In addition, dopaminergic agents such as pramipexole and bupropion produced decreases in immobility time in the forced swimming test (FST) in an experimental model of treatment-resistant depression. Thus, it is hypothesized that dopamine plays a role in certain symptoms underlying depression.

A motivational deficit is one of the more common symptoms of depression. It is also well established that dopamine is involved in some affective behaviors such as motivation for reward. According to the incentive salience hypothesis, dopamine levels in the nucleus accumbens (NAc) are significantly elevated upon presentation of rewarding stimuli, while dopamine becomes more responsive to reward prediction compared to reward acquisition after learning the relationship between reward and predictive cues [2, 3]. Since motivation for reward arises from the certainty of getting rewards, we hypothesized that these changes in dopamine responses represent alterations in reward responsiveness, or motivation. Taken together with the efficacy of dopamine receptor agonists for depression in clinical and pre-clinical studies [1, 4-7] and the contribution of dopamine systems to motivation, it is hypothesized that dopaminergic agents may exhibit antidepressant-like effects by affecting motivational circuitry. Experimental procedures separately evaluating motivational and antidepressant-like effects of drugs may help to reveal the involvement of neurotransmission in motivation. However, the evaluation of this hypothesis has been somewhat restricted since there have only been a few studies investigating the simultaneous motivational and anti-depressive properties of putative therapeutic drugs.

Runway procedures incorporating reward stimulation, such as intracranial self-stimulation (ICSS) of the medial forebrain bundle (MFB) or self-administration of addictive drugs, have been suggested as a useful experimental tool to separately study the reward acquisition process and motivational properties typically associated with ICSS behavior [8-10]. Moreover, our previous study suggested that priming stimulation may regulate motivated behavior in the runway model of ICSS [11]. Since ICSS

measures have been proposed as experimental models for evaluating altered interest or pleasure/hedonia, ICSS has been recognized as a valid measure of motivational state [12]. Indeed, our previous studies demonstrated that both nicotine and methamphetamine enhanced motivated behavior as observed in the runway model of ICSS [13-15]. However, it is not clear whether these behavioral alterations reflect a motivational enhancement for receiving ICSS reward or a reinforcing effect of these addictive drugs. Reward circuitry is reported to be involved in drug addiction as well as motivation for natural rewards [16]. Unfortunately, few studies have simultaneously evaluated motivation and addiction-related behaviors, such as conditioned place preference (CPP) and drug-seeking behavior. Therefore, behavioral experiments discriminating between motivated behavior and addiction-related behavior may facilitate the understanding of precise mechanisms underlying motivation.

In the present study, we evaluated the motivational effect of the dopamine re-uptake inhibitor GBR12909 and the dopamine receptor agonists SKF38393 and quinpirole in the runway model of ICSS to determine the role of dopamine neurotransmission in this animal model. Moreover, we investigated the comparative effects of GBR12909 in the runway ICSS model, forced swimming test (FST), and CPP tests to elucidate behavioral and pharmacological differences between the runway model of ICSS and these other traditional animal models of affective behavior.

2. Materials and Methods

2.1. Animals

In the runway model, male Wistar rats (Charles River, Japan) weighing 250-300 g at the time of surgery were used. Two animals were housed in individual plastic cages (26 × 36 × 25 cm). For FST experiments, male Wistar rats (Charles River, Japan), weighing 180-200 g at the time of testing were used. Three animals were housed in individual plastic cages. In the CPP experiment, male Sprague-Dawley rats (Charles River, Japan) were used and weighed 330-400 g at the start of drug conditioning. Two animals were housed in individual plastic cages. The housing room was maintained at 22 ± 2 °C with an alternating 12-h light/dark cycle (lights on at 19:00). Food and water were provided

ad libitum. Another animal group was used in each treatment group in the experiments. The experimental protocol was conducted according to the Guidelines of the Ethics Review Committee for Animal Experimentation of Okayama University Medical School.

2.2. Drugs

Imipramine hydrochloride, GBR12909 dihydrochloride, (±)-SKF38393 hydrochloride, (-)-quinpirole hydrochloride, haloperidol, S(-)-raclopride (+)-tartrate salt, and (-)-nicotine were used. All drugs were purchased from Sigma Chemical (St. Louis, MO, USA). Imipramine, SKF38393, quinpirole, and raclopride were dissolved in saline (0.9% sodium chloride). Nicotine was dissolved in saline and the pH was adjusted to 7.0 with NaOH. GBR12909 and haloperidol were suspended in 0.5% carboxymethylcellulose. Imipramine, GBR12909, SKF38393, quinpirole, haloperidol, and raclopride were administered intraperitoneally (i.p.) and nicotine was administered subcutaneously (s.c.) with an injection volume of 0.1 ml per 100 g body weight. Administered nicotine dose is expressed in terms of the free base.

2.3. Experiment 1: Assessment of the motivational effect of drugs in the ICSS runway model and involvement of dopamine receptor transmission in this behavior.

2.3.1. Surgery

Animals were anesthetized with an i.p. injection of 50 mg/kg sodium pentobarbital (Somnopentyl®, Kyoritsu Seiyaku, Tokyo, Japan), and stainless steel electrodes comprised of a twisted pair of stainless steel wires (tip diameter: 0.2 mm, insulated except for the top 0.5 mm of the tips) were stereotaxically implanted (SR-5; Narishige, Tokyo, Japan) into the MFB at the level of the posterior hypothalamus of the rat according to The Rat Brain in Stereotaxic Coordinates, 4th ed. (flat skull coordinates: 2.8 mm posterior to bregma, 1.8 mm lateral to the sagittal suture, and 8.5-9.0 mm below the skull surface)[17, 18]. After the electrodes were inserted into the MFB, they were connected to the pins of a small socket (13.95 × 14.5 × 13 mm), which was fixed to the skull using dental cement and two screws driven into the skull. At least 7 days of

recovery were allowed before the onset of training for intracranial self-stimulation behavior in a Skinner box.

2.3.2. Apparatus

A Skinner box (30.8 cm in width, 25.4 cm in length, and 27.7 cm in height) and a runway apparatus (Neuroscience, Tokyo, Japan) were used. The runway apparatus was made from 5 mm acrylic board and consisted of a start box (26.5 cm in width, 26 cm in length, and 30 cm in height) with a controlled start door that opened downward, a runway (18 cm in width, 180 cm in length, and 30 cm in height), and a priming box (30 cm in width, length, and height). A retractable lever (the goal lever) was placed at the end of the runway, 7 cm above the floor. Constant current stimulation in the form of 0.2-ms pulses of 60 Hz alternating current was used for the stimulation. The stimulation current was individually adjusted for each rat.

2.3.3. Experimental procedures

The experiments were performed as previously described [13]. One week after surgery the rats were tested for self-stimulation in the Skinner box. The rats that pressed the lever at a stable rate for three consecutive days in the Skinner box (50 presses per minute) were used for the runway experiment. Each rat was trained in the runway apparatus until its running speed stabilized. Upon reaching the goal end and pressing the lever, they received a reward stimulation (single train of 0.2-ms pulses of 60 Hz alternating current). In a trial, the rat was removed from the runway as soon as it received reward stimulation and was placed in the priming box that stood beside the runway, where 25 seconds later it received 10 priming stimulations (1 stimulation per second with the same parameters as the reward). When priming stimulation ceased, the rat was immediately transferred from the priming box to the start box of the runway. Five seconds after cessation of the priming stimulation, the start box door opened. If the rat ran to the goal lever and pressed it, the rat received a reward stimulation. The current was set at 50-200 μA to produce a maximal priming stimulation effect (PSE; a maximal difference between the running speeds on primed versus unprimed trials). The running

time from the opening of the start door to pressing the goal lever was analyzed via microcomputer on a 0.1-second time scale.

2.3.4. Technique for estimating the motivational effect of drugs in the runway model

This experimental procedure involved 30 trials and consisted of Pretest sessions, Baseline sessions, and Test sessions (Fig 1a). Each session was comprised of 10 trials. In the Pretest session, the rat received 10 priming stimulations and 1 reward stimulation for pressing the goal lever. In the Baseline session, the rats received 5 priming stimulations and 1 reward stimulation for pressing the goal lever. In the Test session, after the administration of drugs, rats received 5 priming stimulations and 1 reward stimulation for pressing the goal lever. Drugs or respective vehicles were administered before the Baseline or Test session. The experimental design for measuring motivation using the runway model is shown in Fig 1a. The running speeds in the Baseline and Test sessions were significantly lower compared to the Pretest session, and were associated with a decrease in priming stimulation frequency (Fig 1b) [$F(2, 15) = 6.407$, $P < 0.01$]. No significant change in the running speed was observed between Baseline and Test sessions. In this experimental model, if administered drugs affect motivation for receiving reward, the running speed in the Test session should be significantly increased or decreased in comparison to the Baseline session (Fig 1c). Therefore, the motivational effect (%) of the drugs was represented as the ratio of the Test session running speed (cm/second) to the Baseline running speed (cm/second). Drugs or vehicle (control) were administered as soon as the Baseline session was finished. GBR12909 (1, 3 and 5 mg/kg), imipramine (5, 10 and 20 mg/kg), and dopamine receptor agonists (SKF38393; 3, 5 and 10 mg/kg, quinpirole; 0.01, 0.1 and 0.5 mg/kg) were administered 30 min before the Test session. Dopamine receptor antagonists (haloperidol; 0.03, 0.05 and 0.1 mg/kg or raclopride; 0.02, 0.05 and 0.1 mg/kg) were administered 60 min before the Test session. The same time was allowed before the Baseline sessions to eliminate the effects of elapsed time on behavior. Five or six rats per group were used for the experiment.

2.3.5. Effect of dopamine receptor antagonists on the effect of GBR12909 in the runway test

This experiment was conducted in a manner similar to the procedure described in section 2.3.4. Doses of haloperidol (0.03 mg/kg) or raclopride (0.02 mg/kg) that had no effect when administered alone were administered 60 min before the Test session, and GBR12909 was administered 30 min before the Test session. Five or six rats per group were used for the experiment.

2.4. Experiment 2: Assessment of the antidepressant-like effects of drugs in the FST.

FST experiments were conducted according to a method previously reported [6]. Black plastic cylinders (height 37 cm, diameter 15.5 cm) containing 20 cm of water at 25°C were used. Two swim sessions were conducted: an initial 13-min adaptation swim and a 6-min test swim 24 h later. The total period of immobility during the 6-min testing period was recorded using the TARGET series/7M analysis program (Neuroscience, Tokyo, Japan). Immobility time was observed 30 min after the injection of GBR12909 or imipramine. Seven or eight rats per group were used for the experiment.

2.5. Experiment 3: Assessment of the place conditioning ability of GBR12909 and nicotine using conditioned place preference (CPP).

The CPP experiment was conducted according to methods previously reported [19].

2.5.1 Apparatus

Place conditioning studies were conducted using an apparatus consisting of a shuttle box (30 cm in width, 60 cm in length, and 30 cm in height) that was made of an acrylic resin board and divided into two equal-sized compartments. One compartment was white with a textured floor and the other was black with a smooth floor to create equally preferred compartments. All sessions were conducted under conditions of normal room illumination.

2.5.2. CPP procedure

Eight rats per group were used for the experiment. The place conditioning schedule consisted of three phases (pre-conditioning, place-conditioning, and post-conditioning tests). Before the conditioning, the pre-conditioning test was performed as follows: the partition separating the two compartments was removed, and rats that had not been treated with either drugs or saline were then placed at the intersection of the two compartments. The time spent in each compartment during a 900-s session was then monitored using a video camera and scored by an experienced researcher. The compartment where an animal spent their time was determined based on the center of the animal's head. After the pre-conditioning test, conditioning sessions [one session/day×3 days for drugs: one session/day×3 days for saline] were performed once daily for 6 successive days as follows: immediately after injection of drugs (0.2 mg/kg s.c. injection of nicotine or 5 mg/kg i.p. injection of GBR12909), these animals were placed in one compartment, which was the opposite side of the box that animals had spent the most time in during the pre-conditioning test, for 50 min. On alternate days, animals that received saline were placed in the other compartment for 50 min. A day after these conditioning sessions, the animal was placed in the test apparatus without any confinements, and then the relative amount of time spent in these compartments was measured. In the post-conditioning test, the animal had the opportunity to move freely around the different compartments. The CPP scores representing the time spent in the drug-paired compartment at the post-conditioning test minus that at the pre-conditioning test were calculated.

2.6. Data analysis

Values are shown as group means with standard errors of the means. The drug session values are expressed as a percentage of the control session values. The results were evaluated statistically using one-way analysis of variance (ANOVA) followed by Sheffé's test or Bonferroni's test. The significance level was set at $P < 0.05$.

2.7. Histology

At the end of experiment 1, all subjects were given an overdose of sodium

pentobarbital and perfused intracardially with saline and 4% paraformaldehyde. Coronal brain sections were generated and were stained with crystal violet to determine the placement of electrodes.

3. Results

3.1. Motivational effects of GBR12909 and imipramine in the runway model of ICSS.

The motivational effects of GBR12909 (1, 3 and 5 mg/kg) and imipramine (5, 10 and 20 mg/kg) are shown in Fig 2. GBR12909 dose-dependently increased running speed in the runway (Fig 2a). Specifically, administration of 5 mg/kg GBR12909 produced a significant increase in running speed [$F\ (3,\ 20) = 6.198$, $P < 0.01$]. In contrast, imipramine decreased running speed in a dose-dependent manner (Fig. 2b). Administration of 20 mg/kg imipramine significantly decreased running speed [$F\ (3,\ 20) = 18.650$, $P < 0.01$].

3.2. Effect of dopamine receptor antagonists in the ICSS runway model and on the enhancing effects of GBR12909.

Fig 3 shows the effect of haloperidol (0.03, 0.05, and 0.1 mg/kg) and raclopride (0.02, 0.05 and 0.1 mg/kg) on the runway running speed of rats that received priming stimulation. Fig 3a shows that 0.1 mg/kg haloperidol significantly decreased running speed on the runway [$F\ (3,\ 20) = 59.317$, $P < 0.01$]. Fig 3b shows that 0.1 mg/kg raclopride also significantly reduced running speed [$F\ (3,\ 20) = 4.002$, $P < 0.05$]. It was notable that the lowest dose of haloperidol and raclopride had no effect on running speed, yet these drugs dose-dependently decreased running speed. Fig 4 shows the effect of pre-treatment of haloperidol (0.03 mg/kg) or raclopride (0.05 mg/kg) on the effect of GBR12909 (5 mg/kg) on running speed. The enhancement of running speed produced by GBR12909 was completely inhibited by pre-treatment with either haloperidol (Fig 4a) or raclopride (Fig 4b) [haloperidol: $F\ (3,\ 20) = 7.695$, $P < 0.01$; raclopride: $F\ (3,\ 19) = 7.489$, $P < 0.05$].

3.3. Effects of dopamine D1-like and D2-like receptor agonists in the runway model of

ICSS.

Fig 5 shows the effect of SKF38393 (3 and 5 mg/kg) and quinpirole (0.01, 0.1, 0.5 mg/kg) on running speed for ICSS in rats that received priming stimulation. Administration of 5 mg/kg SKF38393 significantly decreased running speed (Fig 5a) [$F(2, 15) = 9.331$, $P < 0.01$]. 10 mg/kg SKF38393 also tended to decrease running speed, but this decrease was not significant (data not shown). Quinpirole also decreased running speed in a dose-dependent fashion (Fig 5b). Specifically, administration of 0.1 and 0.5 mg/kg quinpirole significantly decreased running speed [$F(3, 20) = 45.276$, $P < 0.01$]. Fig 6 shows the effect of co-administration of low doses of SKF38393 (3 mg/kg) and quinpirole (0.01 mg/kg). Co-administration of these drugs also produced a significant decrease in running speed [$F(3, 16) = 3.502$, $P < 0.05$].

3.4. Antidepressant-like effects of GBR12909 and imipramine in the FST.

The effects of GBR12909 and imipramine on immobility time in the FST are shown in Fig 7. GBR12909 decreased immobility time in a dose-dependent manner (Fig 7a). In addition, imipramine also significantly reduced immobility time in the FST (Fig 7b). *Post hoc* analysis with Sheffé's test showed that these effects were significant with GBR12909 at a dose of 5 mg/kg, and with imipramine at a dose of 20 mg/kg [GBR12909: $F(3, 32) = 3.6$, $P < 0.05$; imipramine: $F(3, 31) = 4.398$, $P < 0.05$].

3.5. Effect of GBR12909 on place conditioning using CPP.

As shown in Fig 8, the abused drug nicotine produced a significant increase in time spent in the drug-associated environment [$F(2, 21) = 4.171$, $P < 0.05$]. In contrast, GBR12909 did not produce significant place conditioning compared with saline-treated animals.

4. Discussion

The present study demonstrated that GBR12909 produced an increase in running speed in a runway model of ICSS and a decrease in immobility time in the FST without producing place conditioning behavior in the CPP paradigm. The increase in running

speed was completely blocked by pre-treatment with the D2-like receptor-selective dopamine antagonists haloperidol or raclopride. In addition, both dopamine receptor agonists SKF38393 and quinpirole decreased running speed in the runway model of ICSS. Therefore, we were able to distinguish the effects of dopamine re-uptake inhibition on affective behavior and reveal differential effects of dopamine re-uptake inhibition versus dopamine receptor agonism on motivated behavior in ICSS runway studies.

GBR12909 dose-dependently increased running speed in the runway apparatus during the Test session. These results suggest that GBR12909 enhances the PSE used in the runway model of ICSS. In addition, the enhancement of the PSE caused by GBR12909 was inhibited by pre-treatment with haloperidol or raclopride, although these antagonists at the doses used had no effect alone. Previous studies have reported that GBR12909 is a selective inhibitor of dopamine transporters, which results in elevated dopamine levels [20]. Therefore, a facilitation of motivated behavior in the present study would most likely be caused by the effect of GBR12909 on dopamine re-uptake, similar to its enhancement of ICSS behavior as previously reported [21]. It was reported that motivated behavior in the runway procedure of heroin self-administration was not inhibited by haloperidol although runway behavior using methamphetamine self-administration was inhibited by raclopride [8, 22]. Differences in reward may result in the differential effects of dopamine receptor antagonists. In addition to the effects of dopamine receptor antagonists on the facilitation of PSE caused by GBR12909, a single administration of either haloperidol or raclopride dose-dependently decreased running speed during the Test session. Our results suggest that the PSE in this study may be mediated by dopamine neurotransmission, particularly via dopamine D2-like receptors. Therefore, it was theorized that direct stimulation of dopamine receptors using selective agonists might promote the PSE in the runway model of ICSS. To test this hypothesis, we studied the effects of the dopamine D1-like agonist SKF38393 and the D2-like agonist quinpirole in the runway model of ICSS.

However, a single administration of these drugs did not elevate running speed at the Test session and even decreased speed in a dose-dependent manner. Thus, the direct

stimulation of either D1-like or D2-like receptors failed to enhance motivated behavior in the ICSS runway model. Studies have reported that the full efficacy D2 receptor agonist quinpirole dose-dependently reinstated cocaine seeking [23] and elicited relapse to cocaine self-administration in the reinstatement phase [24]. These reports suggest that quinpirole may enhance these drug-related motivational behaviors. However, quinpirole exhibited a suppressive effect in motivated behavior in relation to ICSS electrical reward, suggesting that the motivational behavior evaluated in the present study may reflect different affective properties. Previous research reported that quinpirole was self-administered intravenously in an inverted-U-shape manner in primates (maximal response was observed at doses of 0.01-0.1 mg/kg/injection), suggesting that quinpirole itself may produce rewarding effects [25]. However, quinpirole was reported to facilitate ICSS behavior via a synergistic interaction between dopamine D1-like and D2-like receptors [26]. Therefore, the suppression of the PSE in this study was likely not due to the reduction of reward-acquisition behavior at the goal lever. Quinpirole may promote the maintenance of lever-pressing behavior by potentiating the rewarding effect of electrical stimulation. Thus, quinpirole may attenuate the effect of priming stimulation not related to the lever directly (provided prior to the operant behavior) and suppress goal-directed running behavior even though these are the same stimulations electrically. Indeed, we previously reported that running speed gradually decreased in an experimental extinction study using the priming stimulation of runway ICSS model [27], suggesting that the priming stimulation and the reward stimulation may produce different effects on motivational behavior in this study. Thus, the effect of D2-like receptor stimulation on the present ICSS motivated behavior may be different from that of previously examined behavior even though the enhancing effects of GBR12909 on the runway ICSS model were inhibited by D2-like receptor antagonists.

The co-administration of low doses of SKF38393 and quinpirole produced a significant decrease in running speed during the Test session, although a single administration of these individual drugs had no effect at the tested doses. Therefore, the simultaneous stimulation of D1-like and D2-like receptors may significantly suppress the PSE in the face of activation of both D1-like and D2-like receptors. Consequently,

the enhancement of motivated behavior in this study does not appear to be simply mediated by dopamine receptor activation, although dopamine neurotransmission is considered to play critical roles in this behavior. Complex neurotransmission mediated by these dopamine receptor subtypes may be important for the production of motivated behavior in this animal model.

The role of dopamine in incentive salience may account for these complicated behavioral results. It has been proposed that the phasic (burst) component of dopamine transmission affects learning and motivation in a distinct manner from tonic dopamine activity, and selective impairment of the phasic component induces a selective reduction in specific forms of reward learning [28]. In this study, GBR12909 would increase phasic dopamine transmission as well as tonic dopamine transmission by potentiating the intrinsic dopamine signaling caused by electrical stimulation of the MFB. In comparison, dopamine receptor agonists would stimulate receptors tonically and would not alter the burst-like stimulation of dopamine signaling, at least when administered systemically. We hypothesize that the motivational effect of the priming stimulation, which releases endogenous dopamine and stimulates dopamine receptors in a phasic manner, was blocked because administered dopamine receptor agonists had already occupied dopamine receptors to create the tonic signal. Therefore, we believe that the effect of phasic dopamine receptor stimulation was relatively lowered and that motivation was decreased in the presence of dopamine receptor agonists.

Increases in running speed on the runway observed in the present study may not be a pure expression of enhanced motivation since GBR12909 has also been known to increase locomotor activity in rodents [20]. It would be difficult to differentiate the motivated elevation of running speed from the increase in non-specific locomotor activity since activation of dopamine neurotransmission is involved in locomotor activity [29-32]. However, we believe that the enhancement of motivation in this experiment does not simply reflect increases in locomotor activity. Previous research has reported that quinpirole had no effect on locomotor activity after a single administration of 0.05 and 0.1 mg/kg [33], although in the present study quinpirole decreased running speed in the runway at these doses. We previously reported that

nicotine increases the PSE in the ICSS runway model without affecting locomotor activity [15]. In addition, it was reported that low doses of haloperidol (under 0.1 mg/kg) seem to not significantly affect locomotor activity, especially in combination with GBR12909 or methamphetamine [34-37]. Thus, we consider that the suppression of motivated behaviors in the presence of haloperidol is not due to a reduction of locomotor activity and that changes in running speed in the ICSS runway model (a goal-directed behavior) do not simply reflect the alteration of locomotor activity.

Contrary to the effect of GBR12909, imipramine decreased the PSE in the ICSS runway model. Imipramine enhances noradrenaline and serotonin neurotransmission by inhibiting the noradrenaline and serotonin transporters without affecting dopamine transporters [38]. Thus, it appears that increases in noradrenaline and serotonin neurotransmission do not enhance the PSE in the runway model of ICSS. In addition, previous research has reported that acute injection of the selective serotonin reuptake inhibitor fluoxetine significantly raised brain stimulation reward thresholds in the MFB [39]. Thus, attenuated effectiveness of electrical brain stimulation generated by goal-lever pressing and priming stimulation would suppress motivated behavior since the goal-lever would no longer be as rewarding.

Acute injection of GBR12909 decreased immobility time in the FST as well as enhanced the PSE in the runway model. These data indicate that GBR12909 exhibits antidepressant-like effects in the FST along with enhancing effects on the PSE in the runway model of ICSS. GBR12909 has been reported to inhibit dopamine uptake and not inhibit noradrenaline or serotonin uptake. Dopamine receptor agonists have been used as augmentation therapy for treatment-resistant depression [40-42]. Moreover, in previous studies in which dopamine receptor agonists were administered intracranially, it was demonstrated that the NAc was one of the critical regions for the antidepressant-like effects of dopamine receptor agonists [5, 6]. Thus, GBR12909 may exhibit antidepressant-like effects through modification of dopamine transmission in the NAc.

Both the motivational and the antidepressant-like effects of GBR12909 are thus considered to arise from dopamine re-uptake inhibition in the NAc. However, dopamine

receptor agonism suppressed motivated behavior even though dopamine receptor agonists were reported to improve depression symptomatology in both clinical and preclinical studies [43, 44]. Moreover, imipramine failed to facilitate motivated behavior in the runway ICSS model although imipramine exhibited antidepressant-like effects in the FST. It is assumed that the runway model using ICSS would not be suitable for the evaluation of 'overall' antidepressant-like effects of drugs, and simultaneous assessment of the runway ICSS model and the FST may be useful for the development of new antidepressants that also produce motivational enhancements. In addition, further investigation into the differences between the effects of dopamine re-uptake inhibitors and direct dopamine receptor agonists will be necessary to reveal the differential dopamine neurotransmission related to motivation versus depression. More electrophysiological and neurobiological examinations employing the runway model of ICSS may help to clarify these mechanisms.

In the CPP paradigm, GBR12909 had no effect on the time spent in the drug-related compartment although nicotine produced expected increases in time spent in the drug-paired compartment. These results suggest that GBR12909 does not produce place preference, which is an indication or model of drug dependence. Considering the results observed in the runway model of ICSS, GBR12909 may improve motivational behavior without causing dependence. Moreover, we demonstrate that the motivational effects of drugs as evaluated in the ICSS runway model are quite distinct from the addictive effects of drugs. These results verify previous reports on the effects of nicotine and methamphetamine that reflected the motivational effects of these drugs [14, 15]. Importantly, the dual assessment of the runway model of ICSS and the CPP paradigm may be useful for the development of novel drug treatments for motivational deficits that are non-addicting. Our results suggest that selective dopamine re-uptake inhibition does not induce place preference. Previous findings show that pretreatment with GBR12909 suppresses cocaine self-administration behavior and antagonizes the dopamine-releasing ability of cocaine in rats [45, 46] suggesting that GBR12909 may not be expected to produce place conditioning. In addition, it was reported that dopamine-deficient mice still form a morphine place preference [47] and that

cocaine-induced place preference is still observed in dopamine transporter knockout mice [48]. Thus, it is possible that dopamine elevations may not be necessary for place preference in certain situations although dopamine is closely involved in reward-related behavior.

In conclusion, we demonstrated the following: (1) GBR12909 elevated motivational behavior in the runway model of ICSS, exhibited antidepressant-like effects in the FST, yet did not produce conditioned place preference. These results suggest the potential effectiveness of GBR12909 for the treatment of motivational deficits in depression and further elucidate the behavioral characteristics related to the PSE in the ICSS runway model. (2) Dopamine receptor agonists suppressed motivational behavior in the runway model whereas the motivational effect of GBR12909 in the runway model was inhibited by dopamine receptor antagonists. These results suggest that motivational behavior in the ICSS runway model is not simply mediated by dopamine receptor stimulation. Thus, findings in the present study contribute to the elucidation of mechanisms underlying motivation and further clarify the behavioral pharmacological differences between natural motivation and psychiatric disorders such as depression and drug addiction. Further studies incorporating biochemical procedures with the runway model of ICSS may identify important neurobiological differences between motivation and aberrant affective behaviors related to mental disease.

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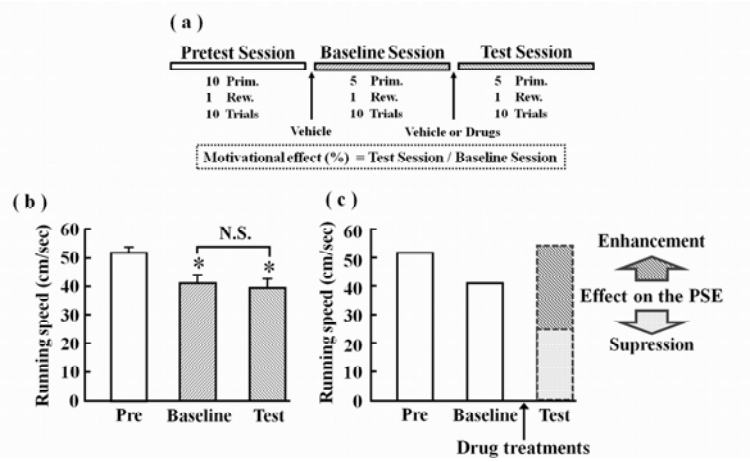
(a)
Pretest Session
Baseline Session
Test Session
10 Prim.
1 Rew.
10 Trials
5 Prim.
1 Rew.
10 Trials
5 Prim.
1 Rew.
10 Trials
Vehicle
Vehicle or Drugs
Motivational effect (%) = Test Session / Baseline Session
(b)
Running speed (cm/sec)
60
50
40
30
20
10
0
N.S.
*
*
Pre
Baseline
Test
(c)
Running speed (cm/sec)
60
50
40
30
20
10
0
Pre
Baseline
Test
Drug treatments
Enhancement
Effect on the PSE
Supression

Fig 1

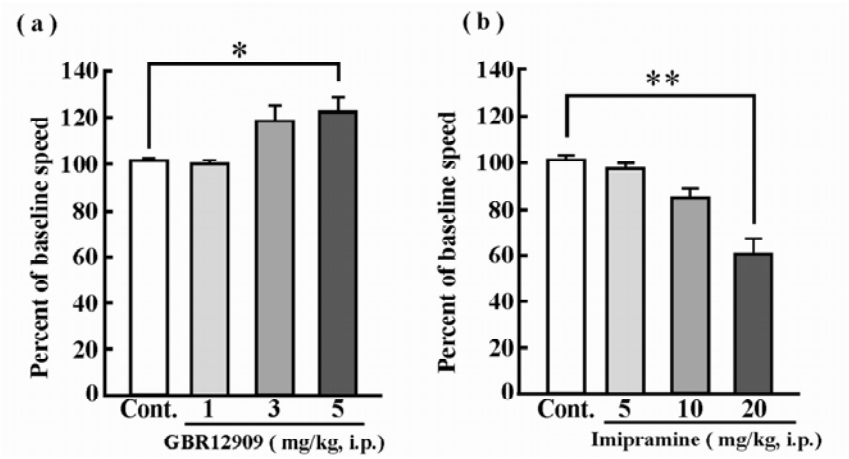
(a)
*
140
120
100
80
60
40
20
0
Percent of baseline speed
Cont.
1
3
5
GBR12909 (mg/kg, i.p.)
(b)
**
140
120
100
80
60
40
20
0
Percent of baseline speed
Cont.
5
10
20
Imipramine (mg/kg, i.p.)

Fig 2

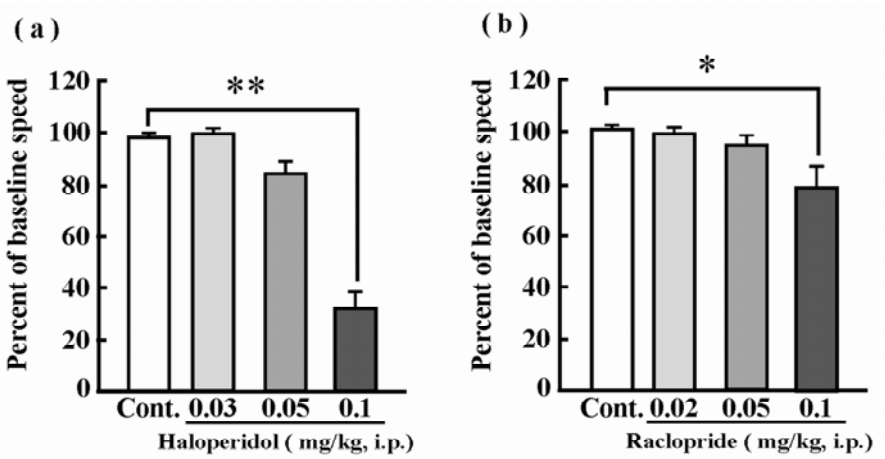
(a)
Percent of baseline speed
120
100
80
60
40
20
0
**
Cont.
0.03
0.05
0.1
Haloperidol (mg/kg, i.p.)
(b)
Percent of baseline speed
120
100
80
60
40
20
0
*
Cont.
0.02
0.05
0.1
Raclopride (mg/kg, i.p.)

Fig 3

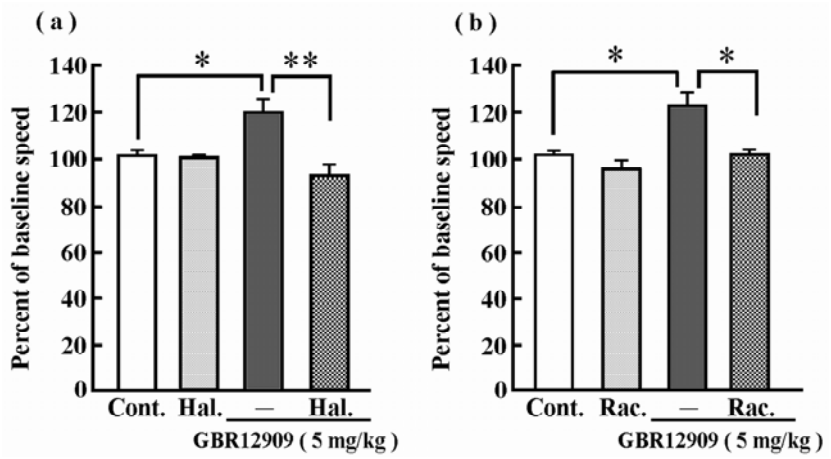
(a)
(b)
Percent of baseline speed
140
120
100
80
60
40
20
0
*
**
*
*
Cont.
Hal.
—
Hal.
Cont.
Rac.
—
Rac.
GBR12909 (5 mg/kg)
GBR12909 (5 mg/kg)

Fig 4

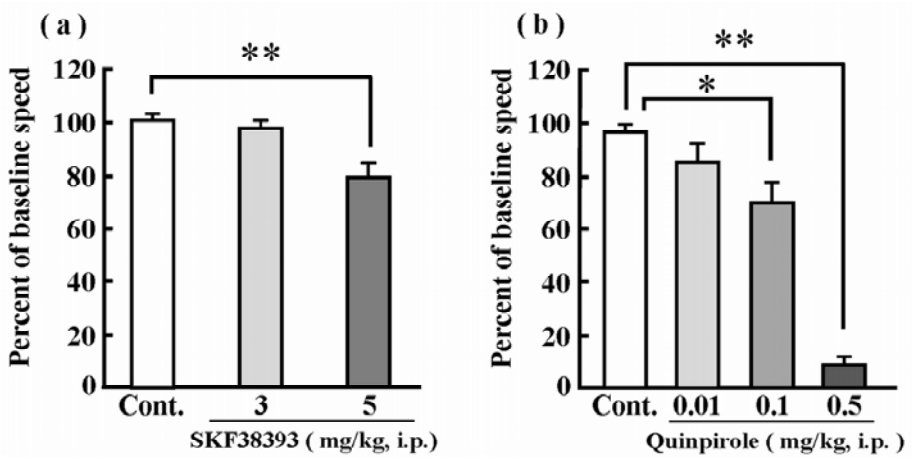
(a)
**
120
100
80
60
40
20
0
Percent of baseline speed
Cont.
3
5
SKF38393 (mg/kg, i.p.)
(b)
**
*
120
100
80
60
40
20
0
Percent of baseline speed
Cont.
0.01
0.1
0.5
Quinpirole (mg/kg, i.p.)

Fig 5

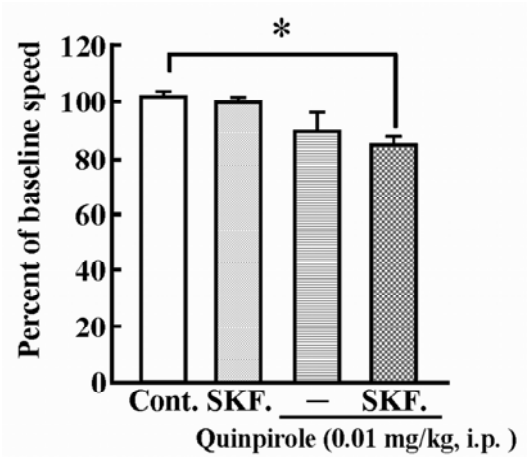
*
120
100
80
60
40
20
0
Percent of baseline speed
Cont.
SKF.
—
SKF.
Quinpirole (0.01 mg/kg, i.p.)

Fig 6

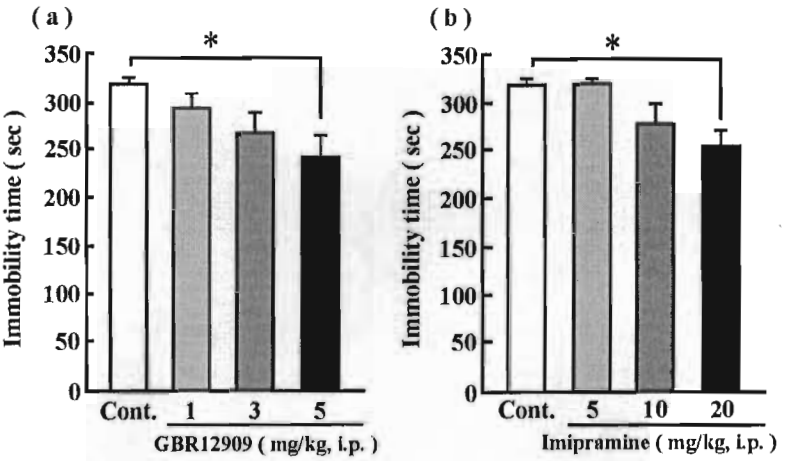
(a)
*
350
300
250
200
150
100
50
0
Immobility time (sec)
Cont.
1
3
5
GBR12909 (mg/kg, i.p.)
(b)
*
350
300
250
200
150
100
50
0
Immobility time (sec)
Cont.
5
10
20
Imipramine (mg/kg, i.p.)

Fig 7

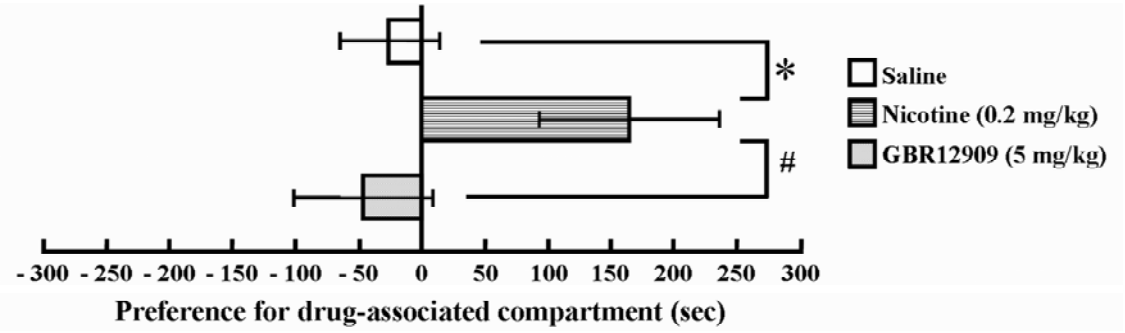
*
#
Saline
Nicotine (0.2 mg/kg)
GBR12909 (5 mg/kg)
- 300 - 250 - 200 - 150 - 100 - 50 0 50 100 150 200 250 300
Preference for drug-associated compartment (sec)

Fig 8

Figure Captions

Fig 1 Experimental design for the measurement of motivational effects and running speed

a: 10 Priming stimulations and 1 Reward stimulation were provided in the Pretest session while 5 Priming stimulations and 1 Reward stimulation were provided in the Baseline and Test sessions. Each session was comprised of 10 trials. The reported value for each rat is the mean value of ten trials in each session. **b**: Running speed for the Pretest, Baseline, and Test sessions. The running speed of the Baseline and Test sessions was measured after the administration of saline. **c**: Schema for the drug effects on the PSE in the runway model of ICSS. If administered drugs enhance the PSE, the running speed should increase (shaded dotted column). Similarly, the running speed should decrease when drugs suppress the PSE (clear dotted column). Each value represents the mean ± S.E.M. of six rats. Data were analyzed by one-way analysis of variance (ANOVA), followed by Scheffé's test. *$P < 0.05$, significantly different from Baseline session. N.S.: Not Significant, Prim.: Priming stimulation.,Rew.: Reward stimulation, Pre: Pretest session, Baseline: Baseline session, Test: Test session.

Fig 2 The motivational effects of GBR12909 and imipramine in the runway model of ICSS

Each column shows the ratio of running speed of the Test session to the Baseline running speed. Data represent the mean ± S.E.M. of six rats. Data were analyzed with one-way analysis of variance (ANOVA) followed by Scheffé's test. **a**: 0.5% carboxymethylcellulose and GBR12909 (1, 3 and 5 mg/kg) were administered intraperitoneally 30 min prior to the Test measurement. Administration of 5 mg/kg GBR12909 produced a significant difference from saline ($P < 0.05$). **b**: Saline and imipramine (5, 10 and 20 mg/kg) were administered intraperitoneally 30 min prior to the Test measurement. Administration of 20 mg/kg imipramine produced a significant difference from saline ($P < 0.01$). Cont.: Control experiment (vehicle administration).

Fig 3 The motivational effects of the dopamine D2-like receptor antagonists haloperidol and raclopride in the ICSS runway model

Each column shows the ratio of running speed of the Test session to the Baseline running speed. Data represent the mean ± S.E.M. of six rats. Data were analyzed with one-way analysis of variance (ANOVA) followed by Scheffé's test. **a**: Saline and haloperidol (0.03, 0.05 and 0.1 mg/kg) were administered intraperitoneally 60 min prior to the Test measurement. Administration of 0.1 mg/kg haloperidol produced a significant difference from saline ($P < 0.01$). **b**: Saline and raclopride (0.02, 0.05 and 0.1 mg/kg) were administered intraperitoneally 60 min prior to the Test measurement. Administration of 0.1 mg/kg raclopride produced a significant difference from saline ($P < 0.05$). Cont.: Control experiment (vehicle administration).

Fig 4 Effects of the dopamine D2-like receptor antagonists haloperidol and raclopride on the PSE-enhancing effects produced by administration of GBR12909 (5 mg/kg) in the ICSS runway model

Each column shows the ratio of running speed of the Test session to the Baseline running speed. Data represent the mean ± S.E.M. of 5-6 rats. Data were analyzed with one-way analysis of variance (ANOVA) followed by Scheffé's test. **a**: GBR12909 was administered intraperitoneally 30 min before the Test session. Haloperidol was administered intraperitoneally 30 min prior to GBR12909. Administration of 5 mg/kg GBR12909 produced a significant difference from saline ($P < 0.05$). Pre-treatment with 0.03 mg/kg haloperidol significantly inhibited the elevation of the motivational effects produced by GBR12909 ($P < 0.01$). **b**: GBR12909 was administered intraperitoneally 30 min before the Test session. Raclopride was administered intraperitoneally 30 min prior to GBR12909. Administration of 5 mg/kg GBR12909 produced a significant increase from saline ($P < 0.05$). Pre-treatment with 0.05 mg/kg raclopride significantly inhibited the elevation produced by GBR12909 ($P < 0.05$). Cont.: Control experiment (vehicle administration) group, Hal.: Haloperidol-treatment (0.03 mg/kg) group, Rac.: Raclopride-treatment (0.05 mg/kg) group.

Fig 5 The motivational effects of the dopamine D1-like receptor agonist SKF38393 and the dopamine D2-like receptor agonist quinpirole in the ICSS runway model

Each column shows the ratio of running speed of the Test session to the Baseline running speed. Data represent the mean ± S.E.M. of 6 rats. Data were analyzed with one-way analysis of variance (ANOVA) followed by Scheffé's test. **a**: Saline and SKF38393 (3 and 5 mg/kg) were administered by intraperitoneal injection 30 min prior to the measurement. Administration of 5 mg/kg SKF38393 produced a significant difference from saline ($P < 0.01$). **b**: Saline and quinpirole (0.01, 0.1 and 0.5 mg/kg) were administered intraperitoneally 30 min prior to the Test measurement. Administration of 0.1 ($P < 0.05$) and 0.5 ($P < 0.01$) mg/kg quinpirole produced a significant difference from saline. Cont.: Control experiment (vehicle administration).

Fig 6 The effect of co-administration of the dopamine D1-like receptor agonist SKF38393 and the dopamine D2-like receptor agonist quinpirole in the ICSS runway model

Each column shows the ratio of running speed of the Test session to the Baseline running speed. Data represent the mean ± S.E.M. of 5 rats. Data were analyzed with one-way analysis of variance (ANOVA) followed by Bonferroni's test. SKF38393 (3 mg/kg) and quinpirole (0.01 mg/kg) were administered simultaneously by intraperitoneal injection 30 min prior to the Test measurement. Co-administration of 3 mg/kg SKF38393 and 0.1 mg/kg quinpirole produced a significant difference from control ($P < 0.05$). Cont.: Control experiment (vehicle administration group), SKF.: 3 mg/kg SKF38393-treatment group.

Fig 7 Effects of a single administration of GBR12909 and imipramine on immobility time during the FST

a: GBR12909 (1, 3 and 5 mg/kg) was administered intraperitoneally 30 min prior to the swim test. **b**: Imipramine (5, 10 and 20 mg/kg) was administered intraperitoneally 30 min before the swim test. Values are expressed as the mean ± S.E.M. for a group of 7-8 rats. Data were analyzed with one-way analysis of variance (ANOVA) followed by

Scheffé's test. *$P < 0.05$, significantly different from the control group. Cont.: Control (vehicle) treatment group.

Fig 8 Effects of nicotine and GBR12909 on place conditioning

Vehicle and nicotine (0.2 mg/kg) or GBR12909 (5 mg/kg) were alternately administered for 6 consecutive days. Place preference was measured after the final administration on day 7. Values are expressed as the mean ± S.E.M. for a group of 8 rats. Data were analyzed with one-way ANOVA followed by Bonferroni's test. *$P < 0.05$, significantly different from the control group. #$P < 0.05$, significantly different from the nicotine-treated group.