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The effects of serotonin and/or noradrenaline reuptake inhibitors on impulsive-like action assessed by the three-choice serial reaction time task: a simple and valid model of impulsive action using rats.

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Abstract

Impulsivity is a pathological symptom in several psychiatric disorders, underscoring the need for animal models of impulsive action to develop a brief screening method for novel therapeutic agents of impulsive action. Our goals were (1) to evaluate whether the 3-choice serial reaction time task (3-CSRTT), a simple version of the 5-choice serial reaction time task (5-CSRTT), is appropriate for brief assessment of impulsive-like action and (2) to examine effects of fluvoxamine, a selective serotonin reuptake inhibitor, and milnacipran, a serotonin/noradrenaline reuptake inhibitor, on impulsive-like action using the 3-CSRTT. Following training in the 3-CSRTT, rats were administered nicotine (0, 0.1, 0.2, and 0.4 mg/kg, salt, s.c.), atomoxetine (0, 0.01, 0.1, and, 1.0 mg/kg, i.p.), fluvoxamine (0, 2, 4, and 8 mg/kg, i.p.), or milnacipran (0, 3, and 10 mg/kg, i.p.). The training time for the 3-CSRTT was significantly shorter than that for the 5-CSRTT. Nicotine increased, while atomoxetine decreased the number of premature responses, an index of impulsive-like action, which is consistent with previous studies. Moreover, we found that milnacipran, but not fluvoxamine, dose-dependently decreased premature responses. These results indicate that the 3-CSRTT could provide an appropriate and simpler rodent model of impulsive-like action and that milnacipran could have some beneficial effects on impulsivity-related disorders.

Keywords: nicotine; atomoxetine; fluvoxamine; milnacipran; impulsivity;
impulsive action; 3-choice serial reaction time task

Introduction

Impulsive acts are often viewed as everyday normal behavior; however, excessive levels of impulsivity are associated with several psychiatric disorders such as attention-deficit/hyperactivity disorder (AD/HD; Solanto, 2002), schizophrenia (Potvin et al., 2003; Enticott et al., 2008), and borderline personality disorder (Paris, 2005). Moreover, it could be a risk factor for drug addiction, criminal involvement, and suicide (Babinski et al., 1999; Corruble et al., 2003; Ohmura et al., 2005; McGirr et al., 2008). In particular, suicide in depressed patients is a major worldwide public health problem. (Kessler et al., 1999; Coryell and Young, 2005). Several studies have demonstrated the relationship between impulsivity and suicide (Baca-Garcia et al., 2005; Raust et al., 2006). Particularly a recent report found the relationship between impulsive action and suicide attempts in bipolar disorder patients (Swann et al., 2005). Therefore, we focused on whether some antidepressants can suppress impulsive-like action. Moreover, further development of preclinical models of impulsive action is required to explore novel agents for treating such impulsivity-related disorders.

Various preclinical models of impulsivity have contributed to understanding of the neural correlates of impulsivity and screening for novel therapeutic agents. In the present study, we focused on the 5-choice serial reaction time task (5-CSRTT), which is one of the most prevalent animal models of impulsive action and is based on the human continuous performance test

(Wilkinson, 1963; Robbins, 2002). This task is performed in an operant chamber containing a horizontal array of five holes. A light in the aperture of one of five holes is briefly and randomly flashed. Animals are required to make a nose-poke response into the flashed hole to get a food pellet. Responses that occur before the presentation of the stimulus light are described as premature responses and resulted in 5 s time-out period. They are regarded as a form of impulsive-like action and hence a failure in impulse control (Robbins, 2002). Thus, premature responses reflect one of the simple forms of impulsive-like action in rodents, so the 5-CSRTT is suitable not only for screening novel treatments but also for revealing the neural basis of impulsive action.

Although this task is useful, it takes a long time to train the animals involved (Puumala et al., 1997). We had speculated that the number of holes is critical factor that determines the time required for the completion of training because the higher the number of holes, the more spatial attention is required. Therefore, we established a 3-choice serial reaction time task (3-CSRTT). We also evaluated the pharmacological validity of the 3-CSRTT as a brief assessment method for impulsive-like action using nicotine, which is well characterized to provoke impulsive-like action in the 5-CSRTT (Blondel et al., 2000; de Bruin et al., 2006; van Gaalen et al., 2006) and using atomoxetine, which is well characterized to suppress that in the 5-CSRTT (Blondeau and Dellu-Hagedorn, 2007; Robinson et al., 2008a).

The evidence that serotonin is profoundly involved in impulse control has been accumulating for a long time (van Praag and Korf, 1974; Robinson et al., 2008b). In the present study, we examined the effects of fluvoxamine, a selective serotonin reuptake inhibitor (SSRI), on impulsive-like action as assessed by the 3-CSRTT. This drug is used as an effective treatment for depression (Vaswani et al., 2003). Although some studies have shown that fluvoxamine suppresses impulsive-like choice in the delay discounting task in rodents (Bizot et al., 1999; Loiseau et al., 2005), whether this effect could be extended to impulsive-like action is unknown. This issue should be addressed because the concept of impulsivity consists of several subordinate concepts (Evenden, 1999a; Mitchell, 1999), and the effects of drugs on impulsive behavior often depend on the type of task employed (Talpos et al., 2006).

The noradrenergic system is also associated with impulsive-like action. Some studies have shown that some selective noradrenaline reuptake inhibitors suppressed impulsive-like choice and impulsive-like action. For example, maprotiline suppressed impulsive-like choice (Bizot et al., 1988) and atomoxetine suppressed both impulsive-like choice/action (Blondeau and Dellu-Hagedorn, 2007; Robinson et al., 2008a). Especially atomoxetine recently came into use for pharmacotherapy of AD/HD. In the present study, we examined the effects of milnacipran, a serotonin/noradrenaline reuptake inhibitor, on impulsive-like action. Given that the beneficial effect of

milnacipran against depression has been established (Ansseau et al., 1989) and that this drug increases both serotonin and noradrenaline in the synaptic cleft (Stahl et al., 2005; Tachibana et al., 2006), it may greatly improve impulse control.

The goals of the present study were (1) to evaluate the validity of the 3-CSRTT as a brief assessment method for impulsive-like action by comparing the training time for the 3-CSRTT with that for the 5-CSRTT and by examining the effects of nicotine and atomoxetine, which have been well characterized in the 5-CSRTT using the 3-CSRTT, and (2) to examine the effects of fluvoxamine and milnacipran on impulsive-like action using the 3-CSRTT.

Materials and Methods

Subjects

Fifty-nine male Wistar/ST rats were supplied by Nippon SLC Co. Ltd. (Hamamatsu, Japan). They were housed in groups of four under an alternating light-dark cycle (light from 7 p.m. to 7 a.m.) at approximately 21°C and relative humidity 40-50%. Twenty-six of fifty-nine rats were used to assess the time required for the completion of training in the 3-CSRTT (n = 14) and the 5-CSRTT (n = 12) (Experiment 1). Following the experiment, the rats were moved to other studies. The thirty-three remaining rats received 3-CSRTT training and were allocated to four experiments: eight

rats were used for testing nicotine administration (Experiment 2), eight rats were used for testing atomoxetine administration (Experiment 3), eight rats were used for testing fluvoxamine administration (Experiment 4), and nine rats were used for testing milnacipran administration (Experiment 5). When the rats were 9 weeks old (270-290 g), we started to restrict their food intake so as to maintain their body weights at 85% of those under free-feeding conditions. The daily foods of rats in the home cage were purchased from CLEA JAPAN, Inc. (CE-2; Tokyo, Japan), and were given after daily session. Their food intakes in the home cage were 10-15 g in the training period and 10-12g (+ palatable food pellets 2-3 g in the operant box, see also 3-CSRTT test procedure) in the experimental period. Water was available ad libitum. The treatment of animals complied with the Guidelines for the Care and Use of Laboratory Animals of the Animal Research Committee of Hokkaido University.

Drugs

(-)-Nicotine hydrogen tartrate salt and (R)-Tomoxetine hydrochloride (atomoxetine hydrochloride) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluvoxamine maleate was purchased from Tocris (Hung road, Bristol, UK). Milnacipran hydrochloride was generously donated by Asahi-Kasei Co. Ltd. (Tokyo, Japan). Nicotine, atomoxetine, and fluvoxamine were dissolved in 0.01 M PBS to adjust the pH of resulting solution to 6.5. These three compounds were administered at a volume of 1 ml/kg.

Milnacipran was dissolved in sterile saline (pH 6.5) and administered at a volume of 3 ml/kg based on our preliminary examination and previous studies (Rénéric and Lucki, 1998; Matsumoto et al., 2005).

Apparatus

Aluminum operant chambers measuring 26 × 26 × 26 cm (Med Associates Inc., St. Albans, VT, USA) were used. The curved rear wall of each chamber contained nine 2.5 cm square holes. Each hole had an infra-red photocell beam for detection of nose-poke responses and a 2.8 W bulb at its rear. Every other hole was sealed so that only the three centrally positioned holes were accessible. A food magazine was located on the opposite wall of the chamber, and a house light was located at the top of this wall. The apparatus was controlled by a computer program written in the MED-PC language (Med Associates Inc., St. Albans, VT, USA).

3-CSRTT and 5-CSRTT training procedure

The same training procedure was employed in the 3-CSRTT and 5-CSRTT. The difference between them was only the number of holes. We employed the same training procedure as Ohmura et al. (2009). In the beginning of the training phase, all hole lights were illuminated for 30 s. In the second phase, one hole light was illuminated randomly for 30 s. In the following phases, the stimulus duration was decreased in a stepwise fashion as the training progressed (stimulus duration 15, 10, 5, 3, 2, 1.5, and 1 s). When a rat

attained the criteria of > 80 % accuracy (percentage of correct responses, see **3-CSRTT test procedure** and **Data analysis**) and < 20 % omissions (percentage of omission errors, see **Data analysis**), it was moved onto the next training phase. The completion of the training was to reach the target phase (stimulus duration 1 s) and to show stable performance for at least three consecutive sessions. Training was conducted for one session per day and six sessions per week.

3-CSRTT test procedure

The task sequence employed in the 3-CSRTT was the same as our previous study using the 5-CSRTT (Ohmura et al, 2009) except for the number of holes. Briefly, when the task started, the house light was illuminated. After a fixed inter-trial interval (ITI: 5 s), one of three hole lights was illuminated randomly and briefly (stimulus duration: 1 s). Nose-poking during the ITI was recorded as a premature response and resulted in turning off all lights (time-out: 5 s), and followed by restarting of the same trial. This parameter was regarded as an index of impulsive-like action. Nose-poking into the lit hole while it was illuminated or within 5 s limited hold was recorded as a correct response and was rewarded by the delivery of a palatable food pellet (45 mg each, dustless precision pellets, Bio-serv, Frenchtown, NJ, USA). As an index of attentional function, accuracy (the percentage of correct responses, see **Data analysis**) was calculated. Nose-poking into another hole was recorded as an incorrect response and resulted in 5 s time-out. The

correct response latency, an index of motor function, and the reward latency, an index of motivation and/or appetite, were also measured. The reward latency was the time between a correct response and nose-poking into the food magazine. When a rat failed to nose-poke within the limited hold, it was recorded as an omission and resulted in a 5 s time-out. This parameter was also regarded as an index of motivation and/or appetite. After a food pellet had been delivered to and collected by a rat, the house light was turned off for 2 s to allow the rat to eat the pellet before the next trial was automatically started. The start of the next ITI was signaled by the turning on the house light. Additional nose-poking into any of the three holes prior to food collection was recorded as a perseverative response and resulted in a 5 s time-out. This parameter was regarded as an index of compulsive behavior. For further analysis, responses to holes during time-out period were also counted though these responses had no consequence. Since the trial was initiated automatically, we did not set a time restriction for this task. Each session consisted of 100 trials.

The current version of the task sequence (Ohmura et al., 2009) differed from the original version (Carli et al., 1983) in some aspects. Premature responses were followed by the restart of the same trial, but not by the start of next trial. Each trial was initiated automatically, not by a panel push. In this way, the rat was required to use more attentional resources (Semenova et al., 2007) because it could not proceed with the task at its own pace.

Furthermore, the stimulus duration was 1 s instead of 0.5 s, because we used Wistar rats, which show poor attention and high impulsive-like action compared to Lister hooded rats (Broersen and Uylings, 1999; Ohmura et al., 2009).

Baseline performance assessment and drug treatment schedule

Once the performance had been stabilized for at least three consecutive sessions, the experiments were started. We used the data from the last 3 days of training to provide a pre-experimental baseline index of performance. Drug treatments were carried out with a Latin square design. The experimental baseline was assessed on Tuesdays and Fridays, and drug administrations were conducted on Wednesdays and Saturdays.

Experiment 1: a comparison of the training time and behavioral parameters in the 3-CSRTT with those in the 5-CSRTT

The training time (sessions) to complete the training in the 3-CSRTT (n = 14) and that in the 5-CSRTT (n = 12) were compared to examine the validity of the 3-CSRTT as a brief assessment method of impulsive action. When the rats achieved stable performance, it was regarded as the completion of the training. We set the criteria for determining stable performance as follows: the change in premature responses stayed within $\pm 25\%$, the accuracy stayed within $\pm 5\%$, and percent response omissions were less than 20% for three consecutive sessions. We also compared these two tasks about the

behavioral parameters such as premature responses, accuracy, percent response omissions, perseverative responses, correct response latency, reward latency, correct responses, and responses during time-out period. Mean value of three consecutive sessions in the stable performance phase was used for each comparison.

Experiment 2: the effects of nicotine on impulsive-like action measured by the 3-CSRTT

Eight rats received an administration of nicotine (0, 0.1, 0.2, and 0.4 mg/kg, salt, s.c.) 10 min before the testing. The doses of nicotine were chosen on the basis of previous studies using the 5-CSRTT (Blondel et al., 2000; de Bruin et al., 2006; van Gaalen et al., 2006).

Experiment 3: the effects of atomoxetine on impulsive-like action measured by the 3-CSRTT

Eight rats received an administration of atomoxetine (0, 0.01, 0.1, and 1.0 mg/kg, i.p.) 30 min before the testing. The doses of atomoxetine were chosen on the basis of previous studies using the 5-CSRTT (Blondeau and Dellu-Hagedorn, 2006; Robinson et al., 2008a).

Experiment 4: the effects of fluvoxamine on impulsive-like action measured by the 3-CSRTT

Eight rats received an administration of fluvoxamine (0, 2, 4, and 8 mg/kg,

i.p.) 30 min before the testing. The doses of fluvoxamine were chosen on the basis of previous studies that assessed impulsive-like choice in rats using the delay discounting task (Bizot et al., 1999; Loiseau et al., 2005).

Experiment 5: the effects of milnacipran on impulsive-like action measured by the 3-CSRTT

Nine rats received an administration of milnacipran (0, 3, and 10 mg/kg, i.p.) 60 min before the testing. The doses of milnacipran were chosen on the basis of a previous study (Rénéric et al., 1998).

Data analysis

Training time and the following eight behavioral measures were analyzed:

- (1) Premature responses (no. per session)
- (2) Accuracy (percentage of correct responses): $[\text{correct responses} / (\text{correct and incorrect responses})] \times 100$
- (3) Omissions (percentage of omission errors): $[\text{omission errors} / \text{total trials}] \times 100$
- (4) Perseverative responses (no. per session)
- (5) Correct response latency (s)
- (6) Reward latency (s)
- (7) Correct responses (no. per session)
- (8) Responses during time-out period (no. per session)

It should be noted that the percentage of omissions is equal to the number of

omissions in the present study because premature response restarted the same trial and we did not set the time limit.

Before the statistical analyses, we conducted Shapiro-Wilk test for all eight behavioral parameters and training periods of both tasks. If we could not assume the normal distribution, the following data transformation were carried out in order to normalize data: percent response omissions were arcsine transformed; premature responses and perseverative responses were subjected to square root transformations; latencies were subjected to customary logarithm transformation. Following that, Mauchly's sphericity test was conducted for analysis of variance (ANOVA) and Levene test for *t*-test. Sphericity assumption and equality of variance were not denied in all measures after transformations. Since training time could not be normalized by transformations, Mann-Whitney U test was conducted for the data. Current graphs were drawn by raw data.

For nicotine, atomoxetine, fluvoxamine, milnacipran dose-response experiments, each measure was analyzed separately by two-factor ANOVA for repeated measures with rank of dose injection as between- and dose as within-subject factor. Multiple comparisons with Bonferroni's correction were also conducted.

Baseline behavioral data were subjected to repeated measures ANOVA.

The alpha level was set at 0.05 for all statistical procedures. All statistical procedures were conducted using SPSS (version 15.0 J).

Results

Experiment 1: a comparison of the time required for the completion of the training and behavioral parameters in the 3-CSRTT with those in the 5-CSRTT

The mean number of sessions necessary to complete the training in the 3-CSRTT was significantly lower than that in the 5-CSRTT (Table 1; 29.93 ± 3.39 vs. 52.58 ± 2.08 , respectively, $z = -3.65$, $p < 0.05$). This analysis indicated that the use of the 3-CSRTT successfully saved about 4 weeks (6 sessions per week) compared with the 5-CSRTT.

Analysis of the behavioral parameters measured by these two tasks (Table 1) revealed that accuracy and correct responses in the 3-CSRTT were significantly higher than those in the 5-CSRTT ($t_{24} = -9.31$, $p < 0.05$; $t_{1,24} = -6.86$, $p < 0.05$, respectively). Moreover, correct response latency in the 3-CSRTT was faster than that in the 5-CSRTT ($t_{24} = 2.85$, $p < 0.05$). Any other parameters did not statistically differ between the two tasks.

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Experiment 2: the effects of nicotine on impulsive-like action measured by the 3-CSRTT

Figure 1a-c show the effects of nicotine on premature responses, percent

correct responses (accuracy), and percent response omissions. The ANOVA revealed a significant dose effect on premature responses ($F_{3, 21} = 17.23, p < 0.05$), but not on accuracy ($F_{3, 21} = 0.35, \text{NS}$) or percent response omissions ($F_{3, 21} = 0.27, \text{NS}$). There is no main effect of rank of dose injection, or significant dose \times rank of dose injection interaction in these three parameters. A multiple comparison with Bonferroni's correction revealed that 0.2 mg/kg dose of nicotine significantly increased the number of premature responses compared to vehicle or the 0.1 mg/kg dose ($p < 0.05$).

The effects of nicotine on perseverative responses, latencies, correct responses, and responses during time-out period were also analyzed (Table 2). Nicotine decreased correct response latency ($F_{3, 21} = 6.77, p < 0.05$) and responses during time-out period ($F_{3, 21} = 3.52, p < 0.05$), but not perseverative responses ($F_{3, 21} = 0.85, \text{NS}$), reward latency ($F_{3, 21} = 0.99, \text{NS}$), or correct responses ($F_{3, 21} = 0.57, \text{NS}$). A main effect of rank of dose injection was observed only in correct response latency ($F_{3, 21} = 10.90, p < 0.05$). There was no significant dose \times rank of dose injection interaction in these five parameters. However, the effects of nicotine on correct response latency and responses during time-out period failed to reach significance in multiple comparisons with Bonferroni's correction though main effects of dose were significant.

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Experiment 3: the effects of atomoxetine on impulsive-like action measured by the 3-CSRTT

Figure 1d-f show the effects of atomoxetine on premature responses, percent correct responses (accuracy), and percent response omissions. The ANOVA revealed a significant dose effect on premature responses ($F_{3, 21} = 12.81, p < 0.05$), but not on accuracy ($F_{3, 21} = 0.58, \text{NS}$) or percent response omissions ($F_{3, 21} = 3.17, \text{NS}$). There is no main effect of rank of dose, or significant dose \times rank of dose injection interaction through these three parameters. A multiple comparison with Bonferroni's correction revealed that 1.0 mg/kg dose of atomoxetine significantly reduced the number of premature responses compared to vehicle ($p < 0.05$).

The effects of atomoxetine on perseverative responses, latencies, correct responses, and responses during time-out period were also analyzed (Table 2). There was no main effect of dose on perseverative responses ($F_{3, 21} = 0.80, \text{NS}$), correct response latency ($F_{3, 21} = 0.14, \text{NS}$), reward latency ($F_{3, 21} = 0.20, \text{NS}$), correct responses ($F_{3, 21} = 0.20, \text{NS}$), or responses during time-out period ($F_{3, 21} = 0.34, \text{NS}$). A main effect of rank of dose injection and significant dose \times rank of dose injection interaction were not observed in these five parameters.

Experiment 4: the effects of fluvoxamine on impulsive-like action measured by the 3-CSRTT

Figure 1g-i show the effects of fluvoxamine on premature responses, percent correct responses (accuracy), and percent response omissions. The ANOVA revealed a significant dose effect on accuracy ($F_{3, 21} = 17.08, p < 0.05$), but not on premature responses ($F_{3, 21} = 0.30, \text{NS}$) or percent response omissions ($F_{3, 21} = 2.19, \text{NS}$). There is no main effect of rank of dose, or significant dose \times rank of dose injection interaction in these three parameters. A multiple comparison with Bonferroni's correction revealed that 8.0 mg/kg dose of fluvoxamine significantly reduced accuracy compared to vehicle or 4.0 mg/kg dose ($p < 0.05$).

The effects of fluvoxamine on perseverative responses, latencies, correct responses, and responses during time-out period were also analyzed (table 2). Fluvoxamine produced a main effect of dose on correct responses ($F_{3, 21} = 9.86, p < 0.05$), but no effect on perseverative responses ($F_{3, 21} = 0.59, \text{NS}$), correct response latency ($F_{3, 21} = 1.45, \text{NS}$), reward latency ($F_{3, 21} = 0.74, \text{NS}$), and responses during time-out period ($F_{3, 21} = 2.01, \text{NS}$). A main effect of rank of dose injection and significant dose \times rank of dose injection interaction were not observed through these five parameters. A multiple comparison with Bonferroni's correction revealed that 8.0 mg/kg of fluvoxamine significantly reduced the number of correct responses compared to the vehicle ($p < 0.05$).

Experiment 5: the effects of milnacipran on impulsive-like action measured by the 3-CSRTT

Figure 1j-1 show the effects of milnacipran on premature responses, percent correct responses (accuracy), and percent response omissions. The ANOVA revealed a significant dose effect on premature responses ($F_{2,16} = 25.43$, $p < 0.05$), but not on accuracy ($F_{2,16} = 1.75$, NS) or percent response omissions ($F_{2,16} = 0.29$, NS). There is no main effect of rank of dose, or significant dose \times rank of dose injection interaction through these three parameters. A multiple comparison with Bonferroni's correction revealed that 10mg/kg dose of milnacipran significantly reduced the number of premature responses compared to the vehicle or 3 mg/kg dose ($p < 0.05$).

The effects of milnacipran on perseverative responses, latencies, correct responses, and responses during time-out period were also analyzed (table 2). There was no main effect of dose on perseverative responses ($F_{2,16} = 0.23$, NS), correct response latency ($F_{2,16} = 1.98$, NS), reward latency ($F_{2,16} = 2.62$, NS), correct responses ($F_{2,16} = 0.48$, NS), and responses during time-out period ($F_{3,21} = 1.81$, NS). A main effect of rank of dose injection and significant dose \times rank of dose injection interaction were not observed in these five parameters.

All eight parameters after vehicle injection were not significantly different among experiments (data not shown, $F_{3,29} < 2$ in all parameters).

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Baseline performance assessment

Figure 2 shows the pre-experimental and experimental baseline and control vehicle administration performance levels in premature responses, accuracy, and percent response omissions for the four groups of rats (nicotine, atomoxetine, fluvoxamine, and milnacipran), over seven (milnacipran) or eight (nicotine, atomoxetine, and fluvoxamine) sessions. Repeated measures ANOVA revealed no significant effects of day on premature responses (nicotine; $F_{7,49} = 1.878$, NS, atomoxetine; $F_{7,49} = 0.89$, NS, fluvoxamine; $F_{7,49} = 1.932$, NS, milnacipran; $F_{6,48} = 2.116$, NS), accuracy (nicotine; $F_{7,49} = 2.165$, NS, atomoxetine; $F_{7,49} = 0.52$, NS, fluvoxamine; $F_{7,49} = 1.932$, NS, milnacipran; $F_{6,48} = 1.079$, NS), or percent response omissions (nicotine; $F_{7,49} = 2.059$, NS, atomoxetine; $F_{7,49} = 1.34$, NS, fluvoxamine; $F_{7,49} = 1.932$, NS, milnacipran; $F_{6,48} = 1.417$, NS) in any of the four groups. This analysis indicated that baseline performance remained stable in all groups during the experiments.

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Discussion

The present study showed that the training time required to reach the criteria (see Materials and Methods) for the 3-CSRTT was significantly shorter than that for the 5-CSRTT. Accuracy and the number of correct responses in the 3-CSRTT were higher than those in the 5-CSRTT. Correct response latency in the 3-CSRTT was faster than that in the 5-CSRTT. Other parameters including premature responses did not differ between the two tasks. Moreover, the administration of nicotine significantly increased the number of premature responses and decreased correct response latency in the 3-CSRTT, which is consistent with previous studies using the 5-CSRTT (Blondel et al., 2000; de Bruin et al., 2006; van Gaalen et al., 2006). Atomoxetine selectively and dose-dependently suppressed impulsive-like action in the 3-CSRTT, which is also consistent with previous reports using the 5-CSRTT (Blondeau and Dellu-Hagedorn 2006; Robinson et al., 2008a). These results indicate that the 3-CSRTT is appropriate as a brief assessment of impulsive-like action in rats. Furthermore, we found that the administration of 10 mg/kg milnacipran significantly suppressed impulsive-like action without affecting any other parameters in the 3-CSRTT while all doses of fluvoxamine failed to alter impulsive-like action in the present study.

Lister hooded rats are often used in the 5-CSRTT. If we had used them in the 3-CSRTT, the duration of training would have been shortened further

because they generally show higher performance in the 5-CSRTT. Nevertheless, we used Wistar strain rats because they are easily handled and show relatively high impulsive-like action (Broersen and Uylings, 1999; Semenova et al., 2007). Also, if the number of holes had been decreased, the duration of training may have been shortened further because a previous report using 2-lever choice task showed that rats moved onto experimental periods only with 24 sessions of training (Evenden, 1999b). However, we chose the 3-hole task because the chance performance level of 2-hole task is high (50%). Moreover, the number of premature responses dramatically increased (> 100) when we used the 1-hole task with Wistar strain rats (unpublished data) as we could not determine whether a rat had nose-poked in response to hole light or had just nose-poked randomly. Decreasing the number of holes may increase the random nose-pokes and data variability (Robbins, 2002) even if we employ 3-hole task. However, in the 3-CSRTT, chance performance level is still 33.33 % and the average of accuracy in the present study was 82.23 % (Table 1), indicating that performance level in the present study was clearly higher than chance level. Although chance performance level in the 5-CSRTT is low (20 %), it should be noted that performance level was also low (the average of accuracy was 63.2 %, see Table 1). Furthermore, there were no significant differences in the number of responses to holes during time-out period and standard error of the mean (SEM) of all behavioral parameters between the 5-CSRTT and 3-CSRTT (see Table 1). Thus, there is no objective evidence indicating that employing the

3-CSRTT increased random nose-pokes and data variability compared to the 5-CSRTT, at least in the present study.

The significant increase of premature responses by the nicotine administration was only observed with the intermediate of the three tested doses. Since the largest dose of nicotine (0.4 mg/kg) also somewhat increased percent response omissions (but not statistically significantly, see Fig. 1c) and significantly reduced responses to holes during time-out period (see Table 2), we suggested that this bell-shaped dose-response curve of premature responses was derived from motor depression. Some previous studies assessing the role for nicotine in locomotor activity with the motor depressant effect in nicotine-naïve rats support this speculation (Clarke and Kumar, 1983; Welzl et al., 1990). The number of premature responses increased by nicotine in the present study was higher than that in previous studies reporting the effects of nicotine in the 5-CSRTT (de Bruin et al., 2006; van Gaalen et al., 2006). Since Blondel et al. (2000) showed that the effects of nicotine on premature responses were higher in rats showing higher baseline of premature responses than those in rats showing lower baseline of premature responses, this result was possibly derived from higher baseline premature responses in the nicotine treated group (see Fig. 2).

Accuracy was not altered in the present study, though some studies reported that the administration of nicotine enhanced attentional performance in the 5-CSRTT (Mirza and Stolerman 1998; Hahn et al., 2002; Bizarro et al., 2004).

This discrepancy may be due to differences in the attentional demands of the task performed between previous studies and the present study. Indeed, Mirza and Stolerman (1998) found a nicotine enhancement effect on attentional performance only when the attentional demands were increased by prolonging the ITI to 20 s, while some other studies found an effect of nicotine on attentional performance under standard tasks (Hahn et al., 2002; Bizarro et al., 2004). Although the 3-CSRTT is appropriate for a brief assessment of impulsive action, its sensitivity for detecting slight changes in attentional function may be somewhat restricted. Further studies will be required to clarify this point.

Atomoxetine dose-dependently suppressed impulsive-like action in the 3-CSRTT, consistent with previous reports using the 5-CSRTT (Blondeau and Dellu-Hagedorn 2006; Robinson et al., 2008a). This indicates that the 3-CSRTT can appropriately detect the decrease of impulsive-like action as well as the increase of that.

While some studies have shown that fluvoxamine suppresses impulsive-like choice in the delay discounting task (Bizot et al., 1999; Loiseau et al., 2005), fluvoxamine did not affect impulsive-like action in the present study. Indeed, it has been suggested that impulsivity consists of several subordinate concepts (Evenden, 1999a; Mitchell, 1999). A growing body of data from preclinical studies has shown that impulsive behavior can be divided into at least two main categories. One is a behavior that result from difficulties in

the ability to inhibit actions, often referred to as “impulsive action”. The level of impulsive-like action could be assessed by using 5-CSRTT and 3-CSRTT. Another is a behavior that reflects impulsive decision making or choice, often referred to as “impulsive choice”. The level of impulsive-like choice could be assessed by using delay discounting task. While there are shared brain areas and receptors responsible for these two behaviors, there are also dissociable brain areas and receptors (Pattij and Vanderschuren, 2008). Therefore, the effects of drugs on impulsive behavior often depend on the type of task employed, possibly because each task assesses different aspects of impulsivity (Talpos et al., 2006).

In the present study, the highest dose of fluvoxamine (8 mg/kg) slightly but significantly impaired accuracy. Since percent response omissions were also somewhat increased (but not statistically significantly, see Fig. 1c), these effects may reflect sedation induced by fluvoxamine. Actually, the number of responses during time-out period was also somewhat decreased (see Table 2). Alternatively, fluvoxamine may impair attentional function, but this effect is not such a critical problem because accuracy was still maintained at a high-level (around 80 %) even at that dose.

The administration of the 10 mg/kg dose of milnacipran, a well-established serotonin/noradrenaline reuptake inhibitor, reduced impulsive-like action in the 3-CSRTT without changing other indices of cognitive function such as attentional function, motivation/appetite, compulsive behavior, or motor

function. These results indicate that milnacipran is a potential therapeutic agent for impulsivity-related disorders. Since our preliminary experiment had shown that a higher dose (30 mg/kg) of milnacipran induced apparent motor depression, we did not use a higher dose (>10 mg/kg) in the present study. Treatments with selective and non-selective noradrenaline reuptake inhibitors such as atomoxetine and desipramine have been shown to produce a reduction in impulsive-like action (Blondeau and Dells-Hagedorn 2006; Robinson et al., 2008a; van Gaalen et al., 2006). Moreover, fluvoxamine, an SSRI, failed to suppress impulsive-like action in the present study. Thus, this suggests that the ability of milnacipran to suppress impulsive-like action is due to its blockade of the noradrenaline transporter, but not of the serotonin transporter. Indeed, previous studies have indicated that alpha-1 and alpha-2 adrenoceptors are involved in impulsive-like action in the 5-CSRTT (Puumala et al., 1997; Koskinen et al., 2003). Nevertheless, it is difficult to conclude that the reduction of impulsive-like action observed in the present study is accounted by an increase in noradrenergic neurotransmission due to the blockade of the noradrenaline transporter because this transporter takes up not only extracellular noradrenaline but also dopamine in some specific brain regions such as the medial prefrontal cortex and the nucleus accumbens shell (Carboni et al., 1990; Pozzi et al., 1994; Yamamoto and Novotney, 1998). Indeed, acute administration of milnacipran increases extracellular concentrations of dopamine in the rat medial prefrontal cortex (Kitaichi et al., 2005). Furthermore, rats with

prefrontal dopamine depletions were found to be involved in impaired impulse control (Sokolowski and Salamone, 1994). Thus, at this time, we cannot determine whether the effects of milnacipran observed in the present study were due to increased noradrenaline or dopamine. To clarify this point, further studies are required.

As stated above, milnacipran suppressed impulsive-like action without affecting other cognitive functions. Moreover, a case report showed that an adult AD/HD patient whose inattention and hyperactivity were remarkably alleviated by chronic administration of milnacipran assessed by continuous performance test (Kako et al., 2007). Our results and this report suggest that milnacipran is not only an antidepressant but could also be a beneficial treatment for impulsivity-related disorders such as AD/HD, schizophrenia, and borderline personality disorder. In addition, milnacipran may lower the risk of suicide in depressed patients, which is a major public health problem.

In conclusion, our present results support the validity of the 3-CSRTT as a brief method for the assessment of impulsive-like action. This preclinical model will contribute to better understanding of the neural basis of impulsive action and help to explore novel treatments for impulsivity-related disorders. We also demonstrated that milnacipran but not fluvoxamine suppresses impulsive-like action without affecting other cognitive functions, indicating that milnacipran could be a beneficial treatment for

impulsivity-related disorders.

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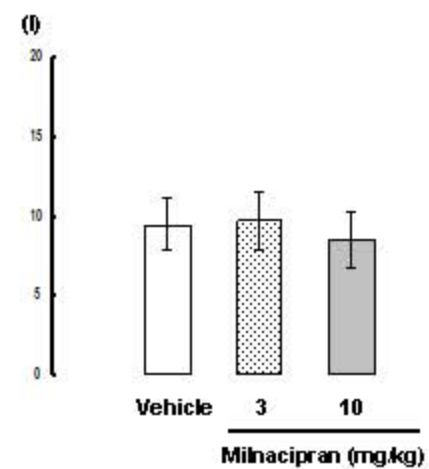
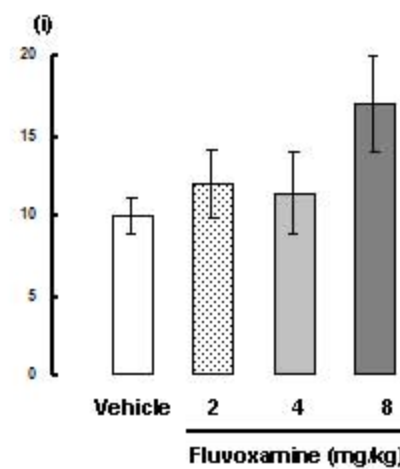
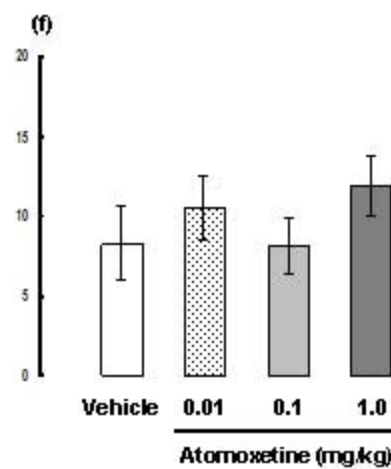
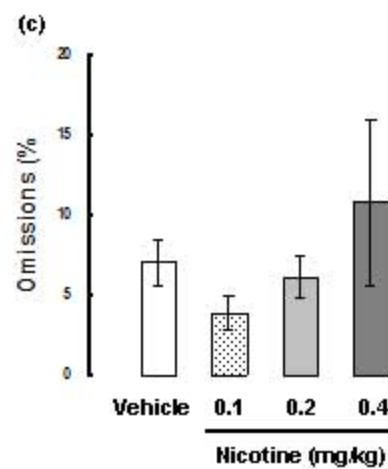
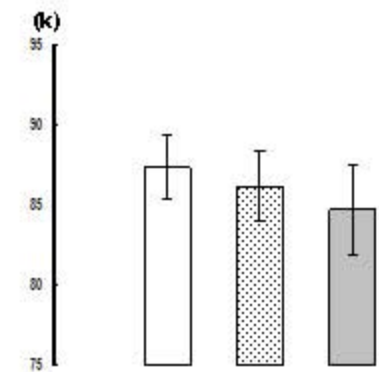
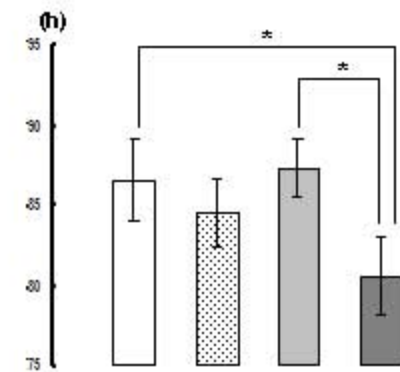
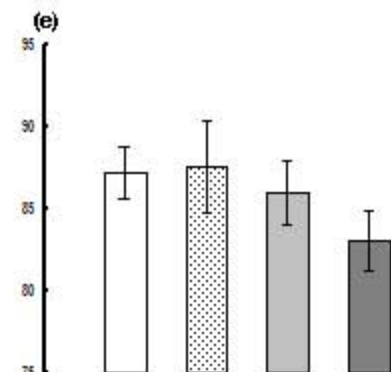
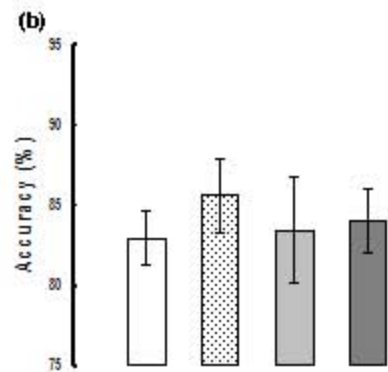
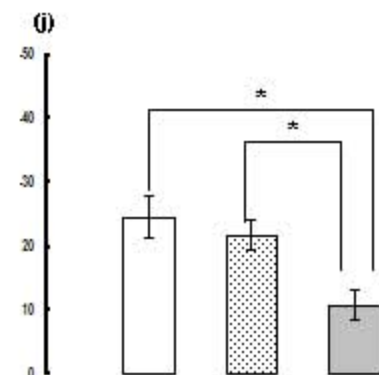
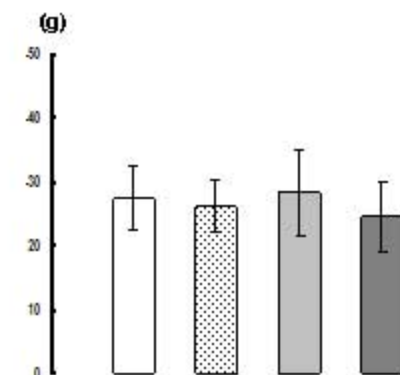
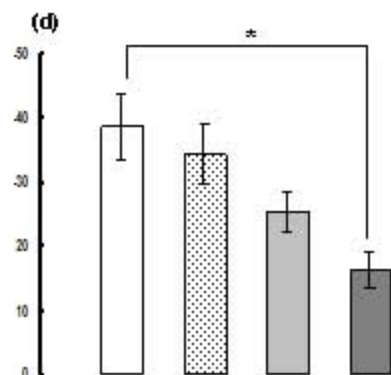
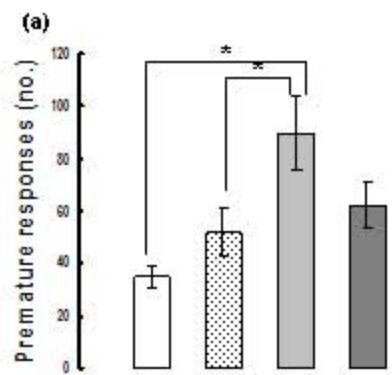
Figure Legends

Fig.1. The effects of nicotine (a-c), atomoxetine (d-f), fluvoxamine (g-i), and milnacipran (j-l) on performance in the 3-CSRTT. Premature responses, accuracy, and percent response omissions are in the columns. Please note that the y-axis scale of premature responses in nicotine treatment group (a) is different from other graphs of premature responses (d, g, i). The bars represent the mean, and the lines represent the SEM.

* $p < 0.05$ (with Bonferroni's correction)

Fig.2. Baseline session

Pre-experimental and experimental baseline and control vehicle administration performance levels of premature responses, accuracy, and percent response omissions in three groups of rats: nicotine, $n = 8$; atomoxetine, $n=8$; fluvoxamine, $n = 8$; milnacipran, $n = 9$. [◆: accuracy (%); ▲: premature responses (no.); ■: percent response omissions (%)]. The lines represent the SEM.



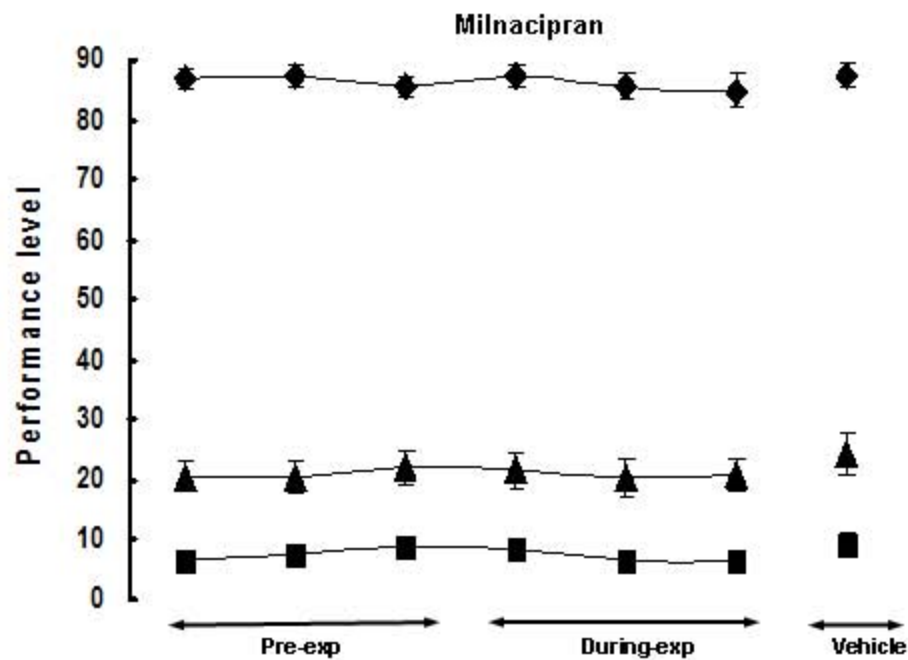
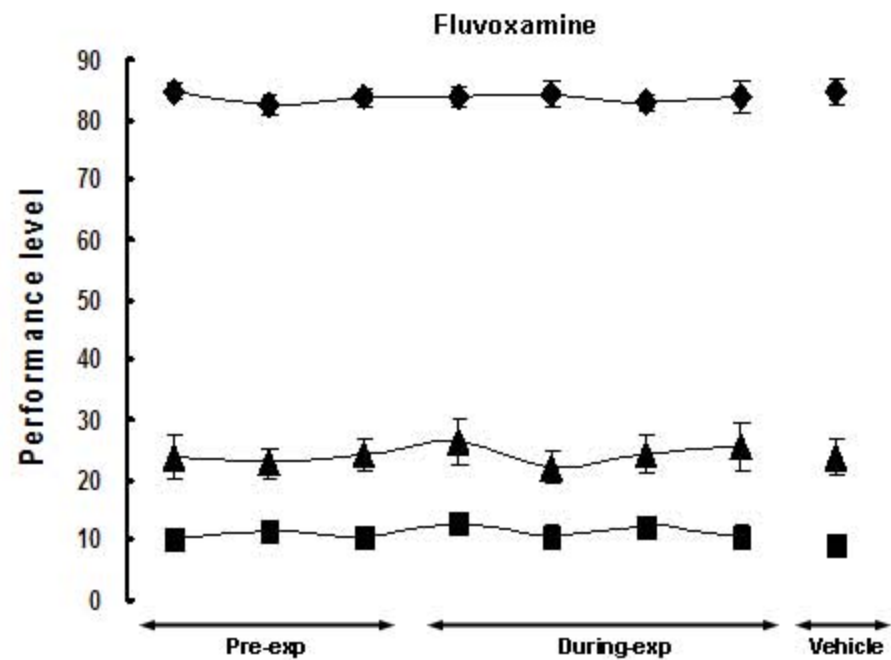
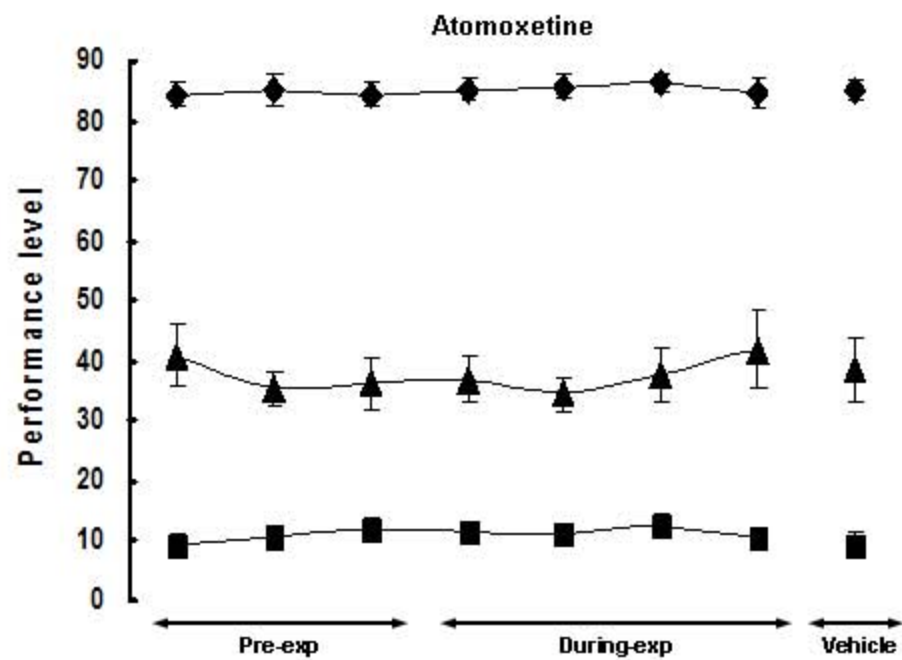
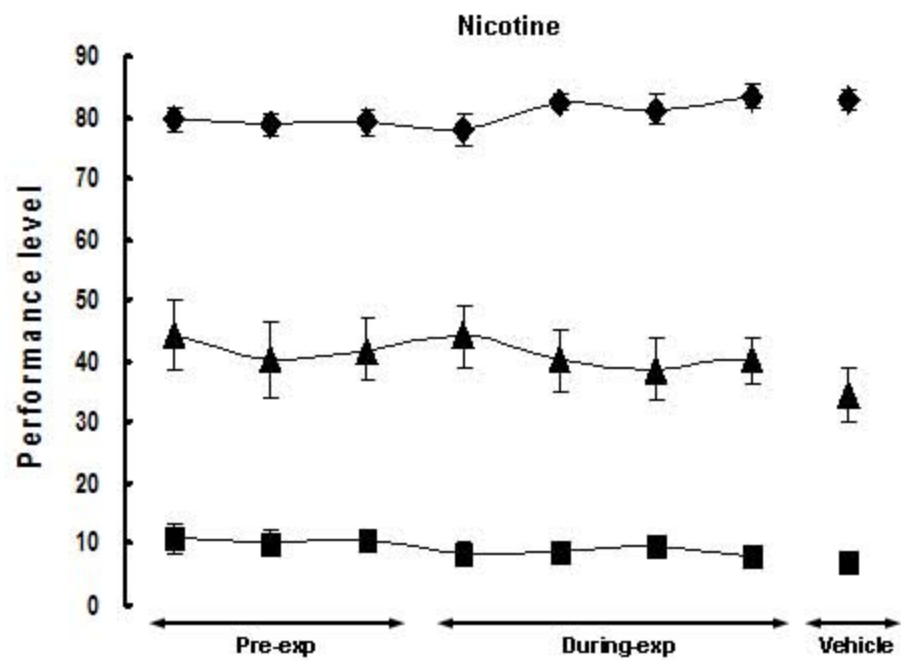


Table 1 A comparison of the duration required for the completion of the training and behavioral parameters in the 3-CSRTT with those in the 5-CSRTT

	3-CSRTT (n = 14)	5-CSRTT (n = 12)
Training time (session)	29.93 ± 3.39*	52.58 ± 2.08
Premature responses (no.)	39.28 ± 5.12	29.33 ± 3.90
Accuracy (%)	82.23 ± 1.19*	63.20 ± 1.33
Omissions (%)	11.05 ± 1.72	11.44 ± 1.24
Perseverative responses (no.)	4.17 ± 0.69	3.47 ± 0.56
Correct response latency (s)	0.59 ± 0.05*	0.77 ± 0.04
Reward latency (s)	1.68 ± 0.05	1.53 ± 0.06
Correct responses (no.)	72.52 ± 6.67*	56.18 ± 5.23
Responses during time-out period (no.)	21.31 ± 8.89	25.81 ± 8.42

Mann-Whitney U test was conducted for the training time while *t*-test was conducted for the other parameters. All data are expressed as mean ± SEM, * $p < 0.05$ versus 5-CSRTT group.

Table 2 Effects of nicotine (n=8), atomoxetine (n=8), fluvoxamine (n=8), and milnacipran (n=9) on perseverative responses, correct response latency, reward latency, correct responses, and responses during time-out period assessed by the 3-CSRTT.

	Peseverative responses (no.)	correct response latency (s)	Reward latency (s)	Correct responses (no.)	Responses during time-out period (no.)
Nicotine (mg/kg)					
Vehicle	4.63±2.09	0.71±0.07	1.74±0.05	77.25±2.37	26.75±10.01
0.1	2.88±2.10	0.69±0.06	1.75±0.08	81.62±2.60	25.13±10.86
0.2	3.50±1.00	0.53±0.04	1.75±0.09	78.50±3.77	28.12±10.65
0.4	3.13±1.20	0.56±0.06	1.70±0.35	74.62±6.33	18.13±7.51
Atomoxetine (mg/kg)					
Vehicle	2.88±0.65	0.54±0.06	1.80±0.08	80.36±3.19	18.25±8.50
0.01	3.63±0.87	0.53±0.06	1.81±0.09	78.90±2.60	17.63±7.65
0.1	3.50±0.67	0.59±0.08	1.83±0.08	79.59±3.58	22.00±9.66
1	3.00±0.91	0.64±0.07	1.95±0.10	73.42±5.20	20.25±10.59
Fluvoxamine (mg/kg)					
Vehicle	3.25±0.94	0.63±0.08	1.79±0.09	78.00±3.10	19.62±10.90
2	4.63±1.48	0.64±0.05	1.87±0.05	74.63±3.41	19.63±10.75
4	3.75±0.68	0.55±0.04	1.85±0.07	77.13±3.41	18.00±9.32
8	3.75±0.41	0.67±0.10	1.90±0.09	64.88±2.57*	11.87±8.90
Milnacipran (mg/kg)					
Vehicle	2.78±0.74	0.56±0.04	1.72±0.05	79.11±2.34	17.33±9.38
3	3.00±0.71	0.68±0.05	1.79±0.05	77.78±2.45	20.67±11.91
10	3.44±0.63	0.67±0.08	1.79±0.03	77.67±3.37	20.89±10.14

Perseverative responses, correct responses, responses during time-out period are the total number per session. Each measure was analyzed separately by two-factor ANOVA for repeated measures with rank of dose injection as between- and dose as within-subject factor. Multiple comparisons with Bonferroni's correction were also conducted.

There were significant main effects of nicotine on correct response latency and responses during time-out period and of fluvoxamine on correct responses. The effect of fluvoxamine, but not nicotine, reached to significance in multiple comparisons with Bonferroni's correction.

All data are expressed as mean \pm SEM. * $p < 0.05$ versus vehicle.