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Title	Milnacipran affects mouse impulsive, aggressive, and depressive-like behaviors in a distinct dose-dependent manner
Author(s)	Tsutsui-Kimura, Iku; Ohmura, Yu; Yoshida, Takayuki et al.
Citation	Journal of pharmacological sciences, 134(3), 181-189 https://doi.org/10.1016/j.jphs.2017.06.004
Issue Date	2017-07
Doc URL	https://hdl.handle.net/2115/67315
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	journal article
File Information	1-s2.0-S1347861317300907-main.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Journal of Pharmacological Sciences 134 (2017) 181–189

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

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

Full paper

Milnacipran affects mouse impulsive, aggressive, and depressive-like behaviors in a distinct dose-dependent manner

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ARTICLE INFO

Article history:
Received 1 May 2017
Received in revised form
12 June 2017
Accepted 14 June 2017
Available online 27 June 2017

Keywords:
Serotonin/norepinephrine reuptake inhibitor
Response inhibition
Violence
Helplessness
Depression

ABSTRACT

Serotonin/noradrenaline reuptake inhibitors (SNRIs) are widely used for the treatment for major depressive disorder, but these drugs induce several side effects including increased aggression and impulsivity, which are risk factors for substance abuse, criminal involvement, and suicide. To address this issue, milnacipran (0, 3, 10, or 30 mg/kg), an SNRI and antidepressant, was intraperitoneally administered to mice prior to the 3-choice serial reaction time task, resident–intruder test, and forced swimming test to measure impulsive, aggressive, and depressive-like behaviors, respectively. A milnacipran dose of 10 mg/kg suppressed all behaviors, which was accompanied by increased dopamine and serotonin levels in the medial prefrontal cortex (mPFC) but not in the nucleus accumbens (NAc). Although the most effective dose for depressive-like behavior was 30 mg/kg, the highest dose increased aggressive behavior and unaffected impulsive behavior. Increased dopamine levels in the NAc could be responsible for the effects. In addition, the mice basal impulsivity was negatively correlated with the latency to the first agonistic behavior. Thus, the optimal dose range of milnacipran is narrower than previously thought. Finding drugs that increase serotonin and dopamine levels in the mPFC without affecting dopamine levels in the NAc is a potential strategy for developing novel antidepressants.

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1. Introduction

Major depressive disorder (MDD) is a major health problem in the world and often leads to suicide.[1] Although selective serotonin reuptake inhibitors (SSRIs) and serotonin/noradrenaline reuptake inhibitors (SNRIs) are widely used for the treatment for MDD, these drugs induce several side effects including increased aggression and impulsivity,[2,3] which are risk factors for substance abuse, criminal involvement, and suicide.[4–8] In contrast, animal studies have shown that SSRIs suppress aggression[9] and that an SNRI and noradrenaline reuptake inhibitor suppress impulsivity.[10–12]

Dose-dependent effects of these drugs might reconcile these contradictory findings because inverted U-shaped dose-response relationships are often observed in psychoactive drugs.[13] Thus, a significant issue concerns whether there is an optimal dose at which an antidepressant suppresses all these behaviors of impulsivity, aggression and depressive symptoms.

There are reliable animal behavioral paradigms that can be used to measure impulsive, aggressive, and depressive-like behaviors, which include the 3-choice serial reaction time task (3-CSRTT),[10] resident-intruder test (RIT),[14] and forced swimming test (FST),[15] respectively. To address above issue, these tests must be conducted in the same animal because food restriction in the 3-CSRTT and isolation in the RIT would alter dose response of drugs.[16,17] Indeed, previous studies have reported that acute milnacipran, an SNRI and antidepressant, reduced the immobility duration in the FST, but the doses at which anti-immobility effects were observed were at least three times higher than those required for anti-impulsive effects in another study.[10,18] However, these behaviors have yet to be examined simultaneously in the same animal study. Therefore, we addressed this issue by using milnacipran and examining the effects on impulsive, aggressive, and depressive-like behaviors in the same animal.

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Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2017.06.004
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To further determine the neural mechanisms underlying the effects of milnacipran on impulsive, aggressive, and depressive-like behaviors, we measured dopamine and serotonin levels in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) following milnacipran administration by using *in vivo* microdialysis. These brain regions and monoamines have been implicated in the modulation of impulsive,[11,19] aggressive,[20] and depressive-like[21] behaviors. Moreover, noradrenaline transporters take up not only noradrenaline but also dopamine from the extracellular space in brain regions including the mPFC.[22]

Additionally, we examined the correlational relationships between impulsive, aggressive, and depressive-like behaviors to explore the potential overlaps in neural mechanisms underlying these behaviors and to determine whether the interrelationships between these psychological components in humans[23] are preserved in animals.

2. Materials and methods

2.1. Subjects

Male C57BL/6N mice supplied by Nippon SLC Co. Ltd. (Hamamatsu, Japan) and bred in our laboratory were used for the 3-CSRTT, RIT (resident), FST, and microdialysis experiments. Male BALB/c mice supplied by CLEA Japan, Inc. (Tokyo, Japan) were used for the RIT (intruder) experiment. They were housed under an alternating light–dark cycle (lights on from 7 p.m. to 7 a.m.) at approximately 21 °C and a relative humidity of 40–50%. The treatment of animals was in compliance with the Guidelines for the care and use of Laboratory Animals of the Animal Research Committee of Hokkaido University.

2.2. Drugs

Milnacipran hydrochloride was generously donated by Asahi-Kasei Co. Ltd. (Tokyo, Japan). The compound was dissolved in 0.9% saline (pH = 6.5–6.8) and administered at a volume of 10 ml/kg.

2.3. 3-Choice serial reaction time task (3-CSRTT)

When the mice were 9 weeks old, they began individual housing and food-restricted diets. Thereafter, their body weights were maintained at 85% of the body weight of mice under free-feeding conditions.

Training and test procedures in the 3-CSRTT have been described in our previous studies using rats.[10,24,25] Detailed procedure in mice was described in supplementary methods because some steps were modified for mice (Supplementary methods).

We used seven behavioral parameters, as described below.

(a) Premature responses (counts per session): a measure of impulsive action.
(b) Accuracy (percentage of correct responses): a measure of attentional function.
(c) Omissions (counts per session): a measure of attentional function and motivation.
(d) Perseverative responses (counts per session): a measure of compulsive behavior.
(e) Correct response latency (s): a measure of attentional function, motivation, and motor function.
(f) Reward latency (s): a measure of motivation and motor function.
(g) Started trials (counts per session): a measure of motivation.

Eight mice received an acute administration of milnacipran (0, 3, 10, or 30 mg/kg, i.p.) 60 min prior to 3-CSRTT testing. Each drug session was conducted with more than a week interval from the previous drug session. The order of the drug injections was counterbalanced using a Latin square design. All 3-CSRTTs were performed between 9:00 a.m. and 11:00 a.m.

2.4. Resident–intruder test (RIT)

After the mice had been individually housed and had been subjected to 3-CSRTT training for 5–6 weeks, they were studied to measure their aggression toward an intruder (Fig. 1). The RIT was conducted as described in Ref. 26. Detailed procedure was described in supplementary methods (Supplementary methods).

Eight resident mice received an acute administration of milnacipran (0, 3, 10, or 30 mg/kg, i.p.) 60 min prior to an encounter with an intruder. Each drug session was conducted with more than a two-day interval from the previous drug session. The order of the drug injections was counterbalanced using a Latin square design. All RITs were performed between 2:00 p.m. and 5:00 p.m.

2.5. Forced swimming test (FST)

Following the completion of the 3-CSRTT testing, 14 mice were subjected to the FST to measure depressive-like behavior (Fig. 1). Thirty-two naïve mice were used for FST drug testing. They received an acute administration of milnacipran (0, 3, 10, or 30 mg/kg, i.p., n = 8 each) 60 min prior to the FST. The FST was conducted as described in Ref. 27. Detailed procedure was described in supplementary methods (Supplementary methods). We used a between-subject design for the FST to avoid repeated exposure to severe stress. All FSTs were performed between 9:00 a.m. and 11:00 a.m.

2.6. In vivo microdialysis and HPLC analysis

Fifty-six mice were used for the HPLC analysis. Mice were anesthetized with isoflurane (Intervet. Inc., Tokyo, Japan) and fixed in a stereotaxic frame (Narishige, Tokyo, Japan). Guide cannulas (AG-4 for the mPFC or CXG-6 for the NAc, Eicom, Japan) were implanted in either the mPFC [AP: +1.9, ML: ±0.2, DV: −0.6] or the NAc [AP: +1.2, ML: ±0.5, DV: −3.25].[28] After surgery, mice were housed individually and allowed a 1-week recovery period before they began testing.

A dialysis probe (for the mPFC, 2-mm long, A-I-4-02, Eicom; for the NAc, 1-mm long, CX-I-6-01, Eicom) was inserted through the guide cannula. The probe was perfused with artificial CSF (2.7 mM KCl, 140 mM NaCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.3 mM NaH_2PO_4, and 1.7 mM Na_2HPO_4, pH 7.2) at a flow rate of 1 μl/min. Mice were placed in plastic observational cages (30 × 30 × 35 cm) and samples were collected every 30 min. Less than 10% variation of the basal monoamine levels was obtained 2–3 h after the insertion of the dialysis probe. Drugs were given by intraperitoneal injection after at least three stable baseline samples were obtained. Dopamine and serotonin concentrations were measured as described previously.[29]

2.7. Data analysis

Behavioral data from the 3-CSRTT and RIT were subjected to a repeated measures analysis of variance (ANOVA) using drug as a within-subject factor. Behavioral data from the FST were subjected to a one-way ANOVA using drug as a between-subject factor. In the microdialysis experiment, the area under the curve (AUC) values for the dopamine and serotonin levels 120 min after the drug

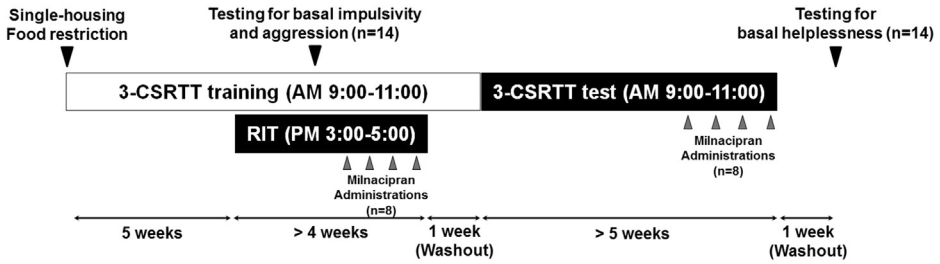


Fig. 1. Experimental schedule. Mice were housed individually and were placed on food-restricted diets when they were 5–9 weeks old. Thereafter, they underwent 3-CSRTT training between 9:00 and 11:00 a.m. Five weeks later, they underwent RIT training between 3:00 and 5:00 p.m. Following the completion of the RIT training, 14 mice were further subjected to encounters for 3 days to determine their basal aggression. Subsequently, eight resident mice received an acute administration of milnacipran (0, 3, 10, or 30 mg/kg, i.p.) 60 min prior to an encounter with an intruder. After a 1-week washout period, one session of 3-CSRTT with a 9-s ITI was conducted to determine their basal impulsivity. Subsequently, eight mice received an acute administration of milnacipran (0, 3, 10, or 30 mg/kg, i.p.) 60 min prior to the 3-CSRTT testing. Following a washout period, 14 mice were subjected to the FST to measure their basal helplessness.

injections were standardized according to the pre-drug baseline values and subjected to a one-way ANOVA using drug as a between-subject factor. Corrections for multiple comparisons with Bonferroni's correction were also conducted after each ANOVA. Pearson's product-moment correlation coefficients were calculated to determine the statistical significance of correlations between basal performances in the 3-CSRTT, RIT, and FST. The α level was set at 0.05 for all comparisons. All statistical procedures were conducted using SPSS (version 15.0 J, IBM, NY, USA).

3. Results

3.1. The effects of acute milnacipran administration on impulsivity in mice

A repeated measures ANOVA revealed a significant main effect of drug on premature response ($F_{3,\ 21} = 9.56$, $P < 0.001$, Fig. 2A) and %omission ($F_{3,\ 21} = 3.34$, $P < 0.05$, Fig. 2C). An intermediate dose of milnacipran (10 mg/kg) significantly reduced the number of premature response compared with the saline. In addition, 30 mg/kg of milnacipran significantly increased %omission compared with the 3 mg/kg of milnacipran. Milnacipran did not affect the other measures ($Fs < 2.06$, *NS*, Fig. 2).

Basal performance levels for premature responses, %accuracy, and %omission remained stable throughout the experiments (Supplemental Fig. S3A–C).

3.2. The effects of acute milnacipran administration on agonistic behavior in mice

A repeated measures ANOVA revealed a significant main effect of drug on attack bite ($F_{3,\ 21} = 8.40$, $P < 0.001$, Fig. 3A), threat behavior ($F_{3,\ 21} = 4.58$, $P < 0.05$, Fig. 3B), agonistic behavior ($F_{3,\ 21} = 10.53$, $P < 0.001$, Fig. 3C), and agonistic latency ($F_{3,\ 21} = 5.16$, $P < 0.05$, Fig. 3D). After using Bonferroni's correction, we detected that 3 mg/kg of milnacipran significantly reduced the number of

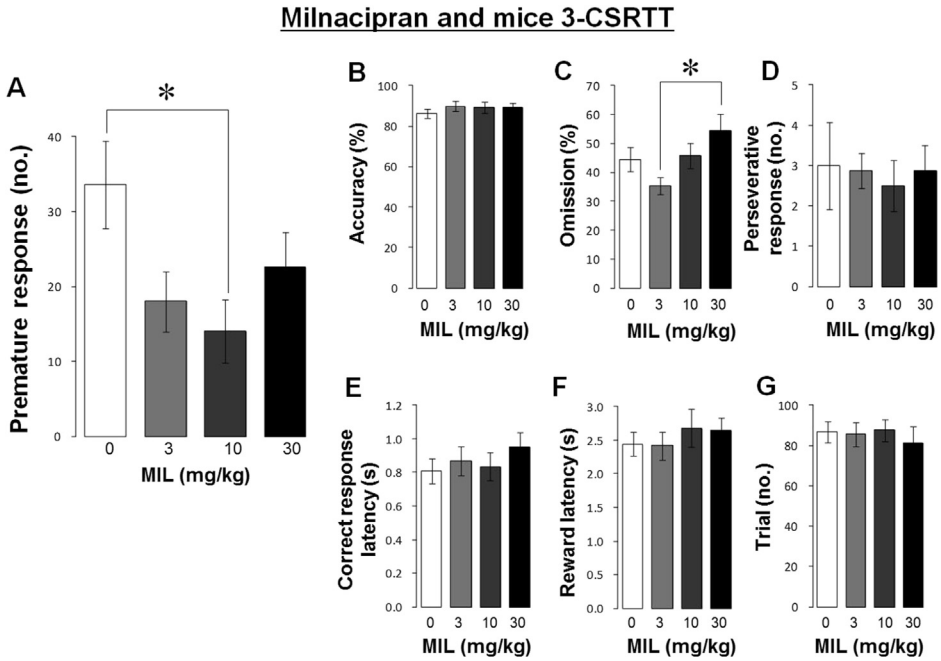


Fig. 2. The effects of acute administration of milnacipran on impulsive behaviors in mice. Milnacipran (0, 3, 10, or 30 mg/kg, i.p.) was administered 60 min prior to the 3-CSRTT. Drugs were administered to the same mice (n = 8) with at least a week interval. The order of the drug injections was counterbalanced using a Latin square design. Milnacipran reduced (**A**) the number of premature responses without changing (**B**) %accuracy, (**C**) %omission, (**D**) perseverative response, (**E**) correct response latency, (**F**) reward latency, and (**G**) the number of trials in the 3-CSRTT. The lines represent the SEM. $^{*}P < 0.05$ with Bonferroni correction (3 mg/kg of milnacipran tended to reduce premature responses, $P = 0.074$).

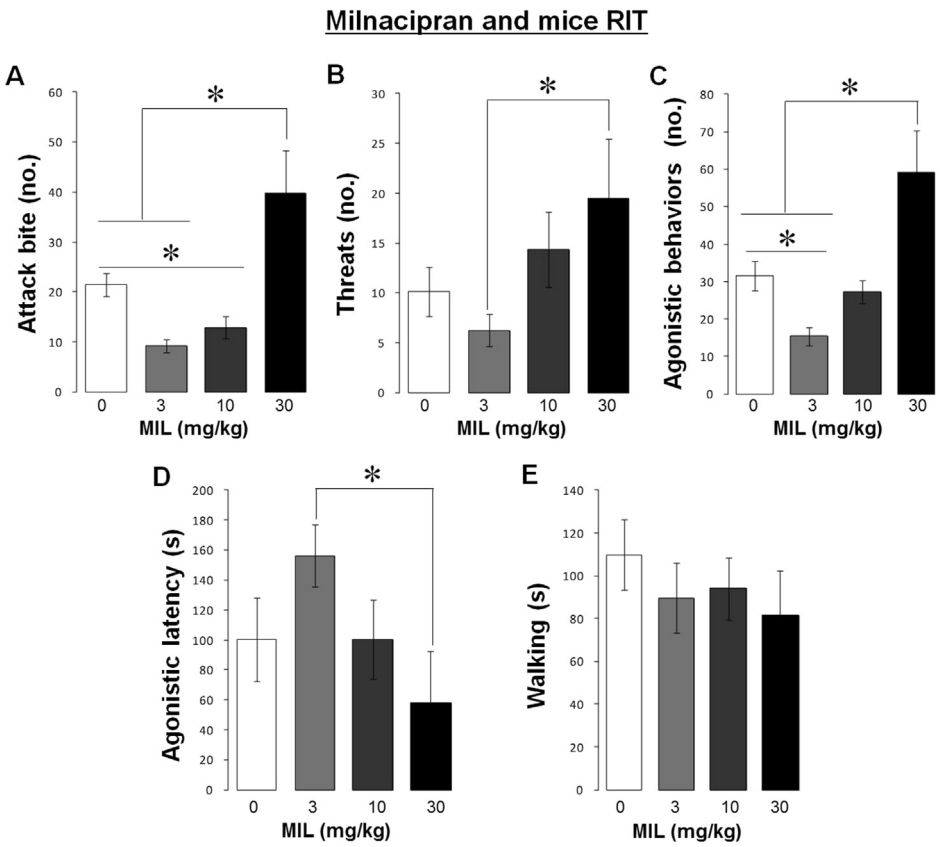


Fig. 3. The effects of acute milnacipran administration on aggressive behaviors in mice. Milnacipran (0, 3, 10, or 30 mg/kg, i.p.) was administered 60 min prior to an encounter with an intruder. Drugs were administered to the same mice (n = 8) with at least a two-day interval. The order of the drug injections was counterbalanced using a Latin square design. Milnacipran reduced (**A**) the number of attack bites, (**B**) threats, and (**C**) agonistic behaviors without changing (**D**) latency to first agonistic behavior and (**E**) walking duration. The lines represent the SEM. *$P < 0.05$ with Bonferroni correction.

attack bites and agonistic behaviors compared with the saline injection; 10 mg/kg of milnacipran significantly reduced the number of attack bites compared with the saline injection. In contrast, 30 mg/kg of milnacipran significantly increased the number of attack bites and agonistic behaviors compared with the saline and 3 mg/kg injections. In addition, 30 mg/kg of milnacipran significantly increased the number of threat behaviors and accelerated the latency to agonistic behavior compared with the 3 mg/kg injection. Milnacipran did not affect the duration of walking ($F_{3, 21} = 0.63$, *NS*, Fig. 3E) at any dose.

Basal performance levels for attack bites remained stable throughout the experiments (Supplemental Fig. S3D).

3.3. The effects of acute milnacipran administration on immobility in mice

A one-way ANOVA revealed a significant main effect of drug on duration of immobility ($F_{3, 31} = 8.90$, $P < 0.001$, Fig. 4A), immobility latency ($F_{3, 31} = 5.62$, $P < 0.01$, Fig. 4B), duration of swimming ($F_{3, 31} = 8.05$, $P < 0.001$, Fig. 4C), and climbing ($F_{3, 31} = 5.06$, $P < 0.05$, Fig. 4D). After using Bonferroni's correction, we detected that 10 and 30 mg/kg of milnacipran significantly reduced the duration of immobility compared with that of the saline group (Fig. 4A). Moreover, 10 mg/kg of milnacipran significantly increased the duration of climbing compared with those of the other three groups (Fig. 4D). In addition, 30 mg/kg of milnacipran significantly prolonged the latency to immobility compared with the other three groups (Fig. 4B) and significantly increased the duration of swimming compared with those of saline and 3 mg/kg groups (Fig. 4C).

3.4. The effects of acute milnacipran on extracellular concentrations of dopamine and serotonin release in the mPFC and NAc of mice

Basal levels of dopamine in 30-min samples were 1.8 ± 0.1 fg/ml in the mPFC and 3.8 ± 0.8 fg/ml in the NAc (mean ± SEM of 28 mice). Basal levels of serotonin in 30-min samples were 1.9 ± 0.2 fg/ml in the mPFC and 2.0 ± 0.3 fg/ml in the NAc (mean ± SEM of 28 mice).

The effects of milnacipran on dialysates of dopamine and serotonin in the mPFC are shown in Fig. 5B–E. A one-way ANOVA revealed significant main effects of the drug on dopamine ($F_{3, 55} = 15.34$, $P < 0.001$) and serotonin ($F_{3, 55} = 31.29$, $P < 0.001$) levels in a time window that was comparable with the phase of the behavioral tests (*i.e.*, 60–120 min after the drug injection). All three doses of milnacipran significantly increased dopamine levels (Fig. 5B and C), and the 10 and 30 mg/kg doses of the drug significantly increased serotonin levels during the time window (Fig. 5D and E).

Fig. 5F–I shows the effects of milnacipran on the dialysates of dopamine and serotonin in the NAc. A one-way ANOVA revealed significant main effects of the drug on dopamine ($F_{3,55} = 29.89$, $P < 0.001$) and serotonin ($F_{3,55} = 15.34$, $P < 0.001$) levels. Only the highest dose of milnacipran significantly increased dopamine (Fig. 5F and G) and serotonin (Fig. 5H and I) levels in the NAc during the time window.

3.5. Correlational analysis: 3-CSRTT vs. RIT/FST

We detected a significant negative correlation between the number of premature responses and latency to agonistic behavior

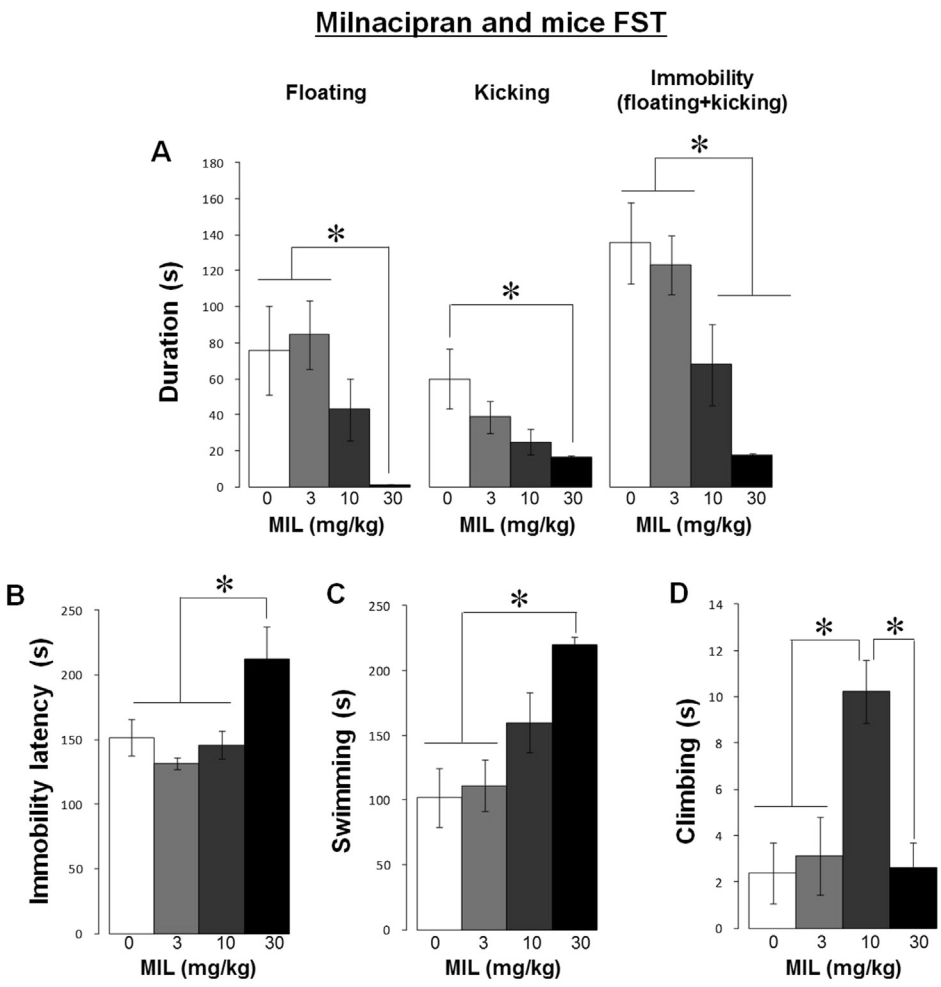


Fig. 4. The effects of acute milnacipran administration on depressive-like behavior in mice. Milnacipran (0, 3, 10, or 30 mg/kg, i.p.) was administered 60 min prior to the FST (n = 8 for each group). Milnacipran reduced (**A**) the duration of immobility but prolonged (**B**) the latency to first immobilization, (**C**) swimming duration, and (**D**) climbing duration. The lines represent the SEM. *$P < 0.05$ with Bonferroni correction.

($r = -0.74$, $P < 0.01$, Fig. 6A), whereas premature responses were not correlated with the number of agonistic behaviors ($r = 0.36$, $P > 0.05$, *NS*, Fig. 6B). There was a significant negative correlation between %omission and the number of attack bites ($r = -0.72$, $P < 0.05$, Fig. 6C) or agonistic behaviors ($r = -0.76$, $P < 0.01$, Fig. 6D). We did not find other significant correlations between behavioral performances in the 3-CSRTT and RIT (Table 1). Furthermore, there was no significant correlation between the behavioral parameters in the FST and those in the other two behavioral tests (Fig. 6E and F and Table 1).

4. Discussion

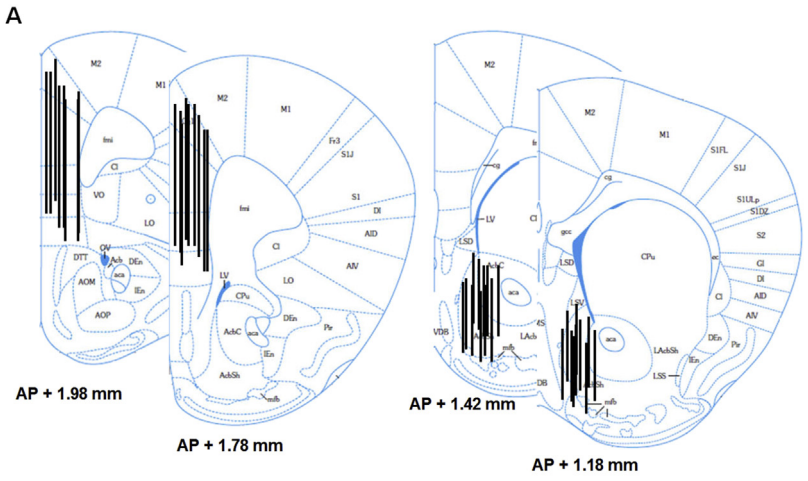
4.1. Effects of milnacipran on impulsive, aggressive, and depressive-like behaviors in mice

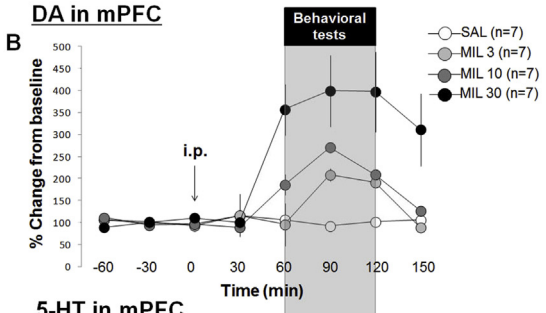
Milnacipran differentially altered impulsive, aggressive, and depressive-like behaviors in a U-shaped, inversely proportional and proportional manner, respectively (Figs. 2–4). We found that only an intermediate dose of milnacipran (10 mg/kg) suppressed all these behaviors (Figs. 2A, 3A and 4A). Although the most effective dose for depressive-like behavior was 30 mg/kg, the highest dose of milnacipran increased aggressive behavior and unaffected impulsive behavior. Thus, unfortunately, we did not find an optimal dose at which milnacipran maximally suppresses all these behaviors of impulsivity, aggression and depressive symptoms.

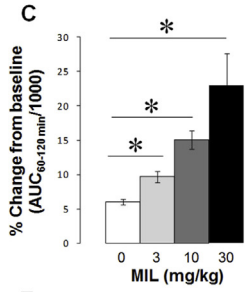
4.2. The potential roles of prefrontal and accumbal dopamine in the effects of milnacipran

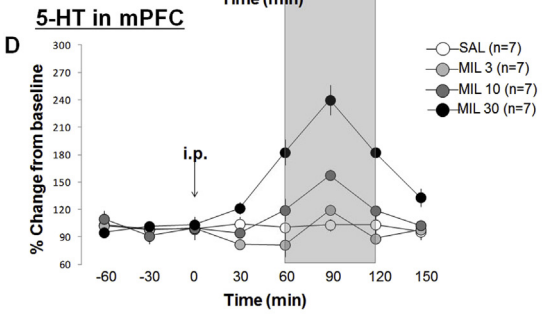
Previous studies reported that milnacipran suppressed impulsive actions by stimulating D_1-like receptors in the ventral mPFC[11] and increased dopamine levels in the NAc provoked impulsive actions.[19] Thus, it is likely that 10 mg/kg of milnacipran reduced impulsive-like premature responses (Fig. 2A) by increasing dopamine levels in the mPFC without changing dopamine levels in the NAc (Fig. 5G) while 30 mg/kg of milnacipran did not suppress impulsive actions because the high dose increased dopamine levels in the NAc.

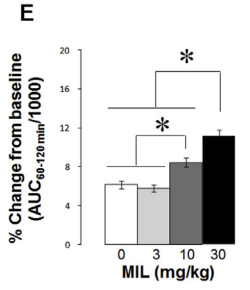
It is difficult to speculate the neural mechanisms by which 30 mg/kg of milnacipran increases dopamine levels in the NAc. Although milnacipran inhibits serotonin/noradrenaline reuptake, it is less likely that milnacipran-induced increase of extracellular noradrenaline levels in the NAc is involved in increased dopamine levels because noradrenergic fibers are sparsely observed in the NAc.[30] It is possible that the blockade of serotonin transporter enhanced dopamine levels in the NAc because previous studies have indicated that serotonin transporter could transport

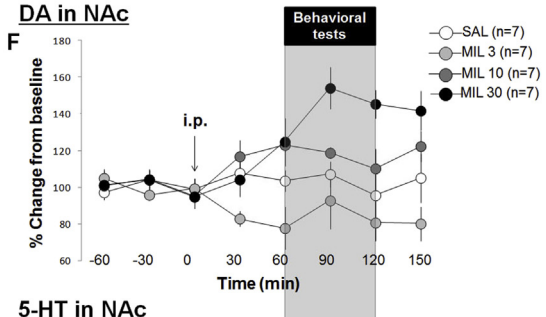

A
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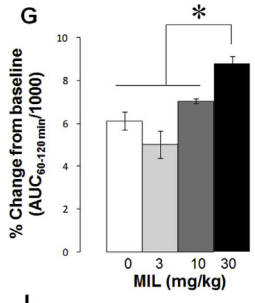

DA in mPFC
B
Behavioral tests
SAL (n=7)
MIL 3 (n=7)
MIL 10 (n=7)
MIL 30 (n=7)
i.p.
% Change from baseline
Time (min)

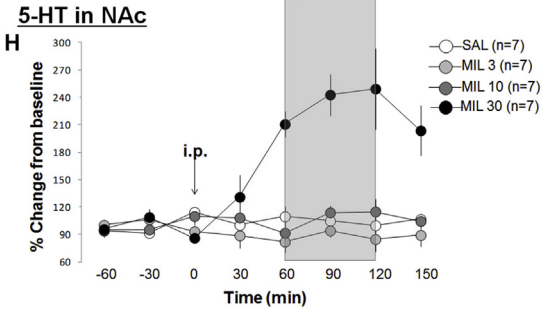

C
% Change from baseline (AUC60-120 min/1000)
MIL (mg/kg)

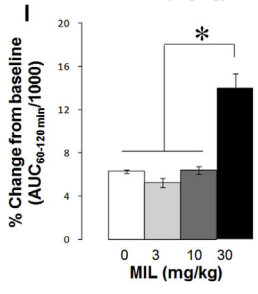

5-HT in mPFC
D
SAL (n=7)
MIL 3 (n=7)
MIL 10 (n=7)
MIL 30 (n=7)
i.p.
% Change from baseline
Time (min)


E
% Change from baseline (AUC60-120 min/1000)
MIL (mg/kg)


DA in NAc
F
Behavioral tests
SAL (n=7)
MIL 3 (n=7)
MIL 10 (n=7)
MIL 30 (n=7)
i.p.
% Change from baseline
Time (min)


G
% Change from baseline (AUC60-120 min/1000)
MIL (mg/kg)


5-HT in NAc
H
SAL (n=7)
MIL 3 (n=7)
MIL 10 (n=7)
MIL 30 (n=7)
i.p.
% Change from baseline
Time (min)


I
% Change from baseline (AUC60-120 min/1000)
MIL (mg/kg)

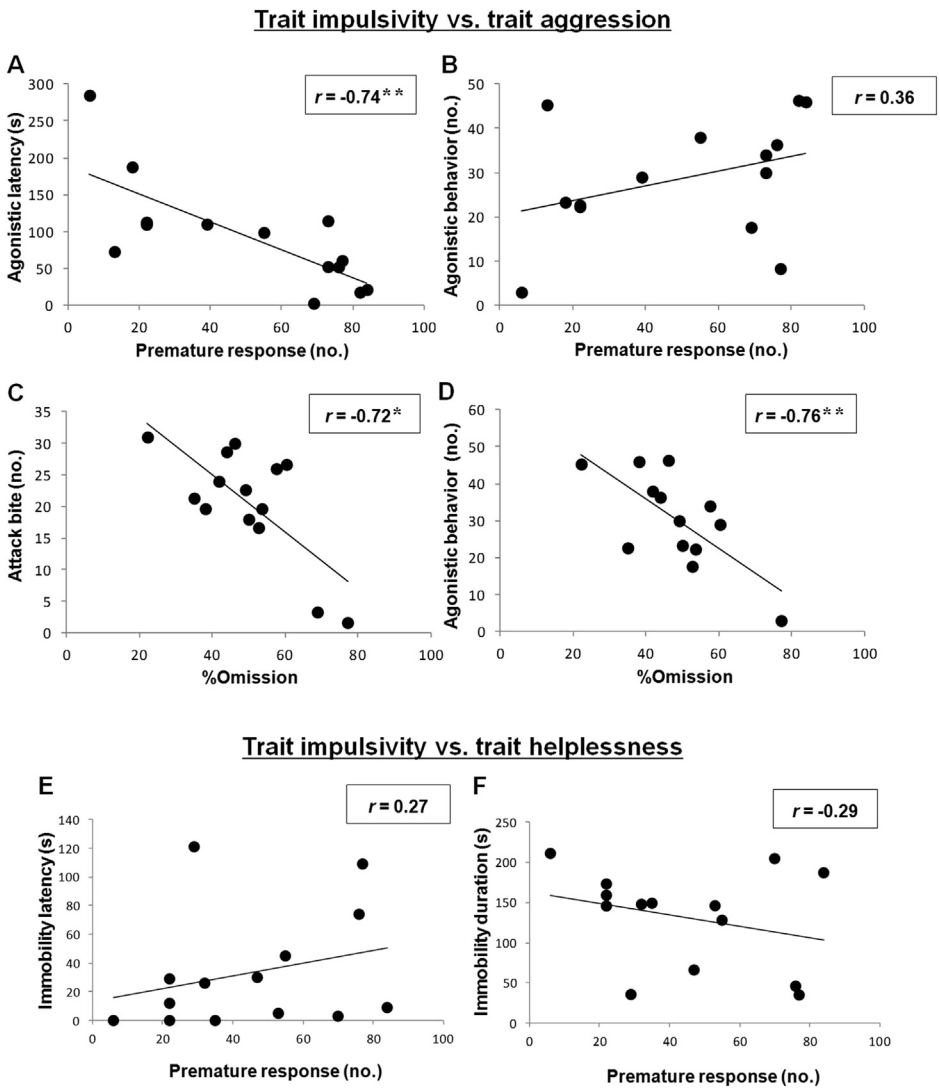


Fig. 6. Scatterplot of impulsive aggressive, and depressive-like behaviors. Pearson's product–moment correlation coefficients among the behavioral parameters recorded in the 3-CSRTT and RIT or FST (**E**–**H**) were calculated for the same animal (n = 14). The number of premature responses was negatively correlated with (**A**) latency to the first agonistic behavior but not with the number of agonistic behaviors (**B**). %omission was negatively correlated with (**C**) the number of attack bites and (**D**) the number of agonistic behaviors. $^{*}P < 0.05$ and $^{**}P < 0.01$.

dopamine in some brain regions where the expression of dopamine transporter is relatively sparse but dopamine levels are higher, such as the NAc shell.[31,32] Indeed, most dialysis probes in the present study were placed in the NAc shell but not in the core (Fig. 5A).

Milnacipran administration also increased serotonin levels in the mPFC (Fig. 5E) and NAc (Fig. 5I). However, a previous study showed that serotonin depletion in the mPFC or NAc unaffected inhibitory control in rats.[33]

The lowest dose of milnacipran (3 mg/kg) showed the highest anti-aggressive effects (Fig. 3). However, it did not affect serotonin levels in the mPFC or NAc, whereas it increased dopamine levels in the mPFC (Fig. 5). To our knowledge, there is no strong evidence that dopamine levels in the mPFC are related to inhibition of aggressive behavior. Thus, it is difficult to speculate the neural mechanisms of anti-aggressive effects of milnacipran by using our data. It is possible that the anti-aggressive effects were partly due to milnacipran-increased serotonin levels in the hypothalamus, which plays a critical role in agonistic behaviors.[34,35]

The highest dose of milnacipran (30 mg/kg) showed pro-aggressive effects (Fig. 3). Increased dopamine levels in the NAc could be responsible for the effects. Dopamine release in the NAc

Fig. 5. The effects of milnacipran on dialysate levels of DA and 5-HT in the mPFC and NAc of freely moving mice. (**A**) Histological verification of the implant sites for the microdialysis probes. Coronal sections drawings of the mouse brain (Franklin and Paxinos, 2007) showing the location of the microdialysis probes in the mPFC (left) and the NAc. The black lines indicate the location of the dialysis in the membrane. Milnacipran dose-dependently increased the levels of cortical dopamine (DA) (**B** and **C**), cortical serotonin (5-HT) (**D** and **E**), accumbal DA (**F** and **G**), and accumbal 5-HT (**H** and **I**) in freely moving mice (n = 7 per group). Shaded areas correspond to the periods in which the three behavioral tests (3-CSRTT, RIT, and FST) were conducted. (**B**, **D**, **F**, **H**) Results are expressed as the mean ± SEM of percentages of baseline values. Arrows indicate intraperitoneal administrations of saline (white circle), 3 mg/kg of milnacipran (light gray circle), 10 mg/kg of milnacipran (dark gray circle), and 30 mg/kg of milnacipran (black circle). (**C**, **E**, **G**, **I**) Data are also expressed as the area under the curve (AUC; mean ± SEM). AUC values are calculated for the amount of DA and 5-HT outflow measured during the 60–120 min post-treatment period with milnacipran and expressed as percentages of the baseline values (n = 7 mice per group). The lines represent the SEM. $^{*}P < 0.05$ with Bonferroni correction.

Table 1
Correlational analyses among the three behavioral paradigms.

Test	Behavioral parameter	Mean ± SEM	Pearson correlation (*r*)			
			VS. Attack bites	VS. Threat behavior	VS. Agonistic behavior	VS. Agonistic latency
3-CSRTT (ITI: 9 s)	Premature response (no.)	50.64 ± 6.69	0.41	0.49	0.36	****−0.74**
	%Accuracy	81.24 ± 2.71	0.20	0.14	0.21	−0.23
	%Omission	49.75 ± 3.73	***−0.72**	−0.53	****−0.76**	0.57
	Perseverative response (no.)	2.29 ± 0.35	0.41	0.47	0.53	−0.05
	Correct response latency (s)	0.98 ± 0.10	−0.02	−0.33	−0.20	−0.20
	Reward latency (s)	2.15 ± 0.19	0.48	0.25	0.45	−0.38
	Total trial started (no.)	74.90 ± 4.83	−0.23	0.10	−0.21	−0.10
RIT	Attack bites (no.)	20.67 ± 2.37				
	Threat behavior (no.)	8.07 ± 1.96				
	Agonistic behavior (no.)	28.74 ± 3.60				
	Agonistic latency (s)	100.13 ± 20.13				
Test	Behavioral parameter	Mean ± SEM	Pearson correlation (*r*)			
			VS. Floating	VS. Kicking	VS. Immobility duration	VS. Immobility latency
3-CSRTT (ITI: 9 s)	Premature response (no.)	50.64 ± 6.69	−0.22	−0.07	−0.29	0.27
	%Accuracy	81.24 ± 2.71	−0.05	0.42	0.23	−0.17
	%Omission	49.75 ± 3.73	0.34	−0.42	0.07	0.14
	Perseverative response (no.)	2.29 ± 0.35	0.57	−0.13	0.52	−0.38
	Correct response latency (s)	0.98 ± 0.10	0.01	−0.07	0.04	−0.21
	Reward latency (s)	2.15 ± 0.19	0.02	−0.14	0.08	0.14
	Total trial started (no.)	74.90 ± 4.83	0.10	0.36	0.36	−0.57
FST	Floating (s)	89.30 ± 16.56				
	Kicking (s)	41.70 ± 10.71				
	Immobility duration (s)	131.0 ± 15.17				
	Immobility latency (s)	33.10 ± 10.09				
RIT	Attack bites (no.)	20.67 ± 2.37	−0.51	−0.50	−0.52	−0.08
	Threat behavior (no.)	8.07 ± 1.96	0.03	0.09	0.09	−0.03
	Agonistic behavior (no.)	28.74 ± 3.60	−0.31	−0.41	0.44	−0.07
	Agonistic latency (no.)	100.13 ± 20.13	0.51	0.42	0.45	−0.12
FST	Floating (s)	89.30 ± 16.56				
	Kicking (s)	41.70 ± 10.71				
	Immobility duration (s)	131.0 ± 15.17				
	Immobility latency (no.)	33.10 ± 10.09				

Note: $^{*}P < 0.05$, $^{**}P < 0.01$ with Pearson's correlation coefficients (n = 14).

was increased during the display of aggressive behavior.[36] Moreover, local infusion of an antagonist for the D_2 receptor into the NAc significantly reduced aggression in rats.[37]

Milnacipran-increased serotonin levels in the mPFC and dopamine levels in the NAc would be responsible for the antidepressant effects (Figs. 4 and 5). It is widely accepted that increased serotonin levels in the mPFC and dopamine levels in the NAc are involved in the antidepressant effects.[21,38,39]

4.3. Interrelationships between impulsive, aggressive, and depressive-like behaviors in mice

The present study is the first to examine correlational relationships between impulsive actions and agonistic behaviors or depressive-like behavior within the same mice. We demonstrated that impulsive-like premature responses assessed in the 3-CSRTT were negatively correlated with the latency to the first agonistic behavior, but not the number of agonistic behaviors measured in the RIT (Fig. 6), consistent with a human study showing that impulsivity affects the decisions of whether to launch an attack but not the number of aggressive attacks.[23] Although mice showed anti-impulsive and anti-aggressive effects with milnacipran treatment, we did not identify a significant correlation between these effects (Supplemental Fig. S4), suggesting the dissociable mechanisms of action underlying the anti-impulsive and aggressive effects of milnacipran. Our results suggest that both impulsivity and aggression assessed by the 3-CSRTT and RIT are comparable with human impulsivity and aggression.

Agonistic behavior also negatively correlated with %omission (Fig. 6), which is an index of sustained attention or motivation. However, another measure of attentional function (%accuracy) or of motivation (the number of total trial started) did not correlate with agonistic behavior (Table 1). Thus, further studies are required to clarify this issue though sustained attention might be required for persistent agonistic behavior.

4.4. Clinical implications and future directions

Considering our current and previous results, the use of a high dose of antidepressants might increase the incidence of aggression, which is a risk factor for suicide.[4,6] Although further studies are required to extrapolate our findings in animals to humans, our current results suggest that clinicians need to find the optimal dosage of antidepressants with due regard for the side effects of aggression because the optimal dose range of antidepressants seems to be narrower than previously thought.

In contrast, low doses of antidepressants would be sufficient to suppress impulsivity and aggression. Low doses of antidepressants might be promising candidates for treating patients with increased aggression and impulsivity such as borderline personality disorder.

Moreover, therapeutic effects were accompanied by increased serotonin and dopamine levels in the mPFC, but not in the NAc.[40] Finding drugs that increase serotonin and dopamine levels in the mPFC without affecting dopamine levels in the NAc is a potential strategy for developing novel antidepressants.[41]

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by a Grant for Research Fellows of the Japan Society for the Promotion of Science 234134 (I.TK.), a Grant-in-Aid for Scientific Research on Innovative Areas to Y.O. [12023490], and a Grant-in-Aid for Young Scientists (A) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) to Y.O. [25713043].

We thank Aki Takahashi, PhD for technical support regarding the resident-intruder test.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jphs.2017.06.004.

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