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Effects of nociceptin on the exploratory behavior of mice in the hole-board test

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Abstract

The effects of nociceptin on the exploratory behavior of mice were examined using an automatic hole-board apparatus. A low dose of nociceptin (0.01 nmol, i.c.v.) had an anxiolytic effect, as reflected by an increase in head-dipping behavior. However, high doses of nociceptin (1–5 nmol, i.c.v.) produced a dose-dependent anxiogenic effect, as reflected by a decrease in head-dipping behavior. Both the anxiolytic and anxiogenic effects of nociceptin were antagonized by nocistatin, an opioid receptor-like 1 (ORL1) receptor antagonist. Although a low dose (0.01 nmol, i.c.v.) of nociceptin significantly increased the rate of serotonin (5-hyroxytryptamine, 5-HT) turnover in the hippocampus, a high dose (5 nmol, i.c.v.) of nociceptin significantly decreased this turnover in the amygdala. Furthermore, the anxiolytic effect of nociceptin at a low dose was antagonized by *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide 3HCl (WAY100635), a 5-HT_{1A} receptor antagonist. On the other hand, the anxiogenic effect of nociceptin at a high dose was antagonized by *R*(+)-2-dipropylamino-8-hydroxy-1,2,3,4–tetrahydronaphthalene hydrobromide (8-OH-DPAT), a 5-HT_{1A} receptor agonist. In conclusion, the results of this study suggest that nociceptin has dose-related anxiolytic and anxiogenic effects as a result of the activation of ORL1 receptors. The present results also suggest that a low dose of nociceptin has an anxiolytic effect via the activation of 5-HT ergic function in the hippocampus, while a high dose of nociceptin has an anxiogenic effect via the inhibition of 5-HT ergic function in the amygdala.
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Keywords: Nociceptin; Nocistatin; Anxiogenic effect; Anxiolytic effect; Hole-board; 5-HT_{1A} receptor

1. Introduction

Nociceptin/orphanin FQ (nociceptin) was identified as a putative endogenous ligand of an opioid receptor-like 1 (ORL1) receptor that has negligible affinity for μ-. δ-and κ-opioids (Mollereau et al., 1994; Nishi et al., 1997). It has been reported that nociceptin induced anxiolytic effects in several animal models of anxiety (light–dark preference, elevated plus maze test, operant conflict) when it was administered intracerebroventricularly (Jenck et al., 1997). Those authors also reported that Ro-64-6198, a selective ORL1 receptor agonist, has dose-dependent anxiolytic-like effects in animal models of anxiety (Jenck et al., 2000). Furthermore, Koster et al. (1999) reported that nociceptin-deficient mice exhibit exaggerated anxious behavior when exposed to a novel environment. These studies indicated that nociceptin plays an important role in regulating the emotional state in rodents. It is also well known that nociceptin influences nociceptive transmission. Interestingly, when nociceptin is administrated intrathecally, it appears to have dual effects consisting of hyperalgesia/allodynia at low doses and clear antinociceptive effects at higher doses (Inoue et al., 1999; Sakurada et al., 1999). However, there have been no previous studies on the dose-related effects of nociceptin on the emotional state in rodents.

Serotonin (5-HT) has been widely assumed to be involved in anxiety (Murphy, 1990). In general, new drugs for anxiety are almost all associated with 5-HT neurotransmission. The identification and characterization of multiple 5-HT binding sites in brain tissues and the synthesis of selective ligands for these receptors in the mid-1980s were

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the starting points for numerous studies on the behavioral effects of 5-HT related drugs in animal models of anxiety. The findings of these studies support the hypothesis that defects in brain 5-HT neuron systems are closely related to anxiety disorders. On the other hand, several investigators have reported that the ORL1 receptor may play an important role in the control of 5-HT ergic neuronal function in the central nervous system (Roumy and Zajac, 1999; Siniscalchi et al., 1999; Sbrenna et al., 2000). Siniscalchi et al. (1999) reported that nociceptin inhibited [^{3}H] 5-HT release from rat cerebral cortex slices. Sbrenna et al. (2000) also reported that the ORL1 receptor agonist nociceptin inhibited both spontaneous and K^+-evoked [^{3}H] 5-HT efflux. Thus, they suggested that nociceptin may play a role in the presynaptic modulation of 5-HT release. Thus, it is possible that emotional changes produced by nociceptin may alter 5-HT neurotransmission.

The hole-board test, which was first introduced by Boissier and Simon (1962, 1964) provides a simple method for measuring the response of an animal to an unfamiliar environment. Previously, the hole-board test has been used to assess emotionality, anxiety and/or responses to stress in animals (Rodriguez et al., 1987). Some advantages of this test are that several behaviors can be readily observed and quantified, which makes possible a comprehensive description of the animal's behavior. Several investigators have previously examined the effects of benzodiazepine anxiolytics such as diazepam on behavior in the hole-board test. However, the results from previous investigations are controversial, i.e., exploratory behaviors were either increased (Nolan and Parkes, 1973; Crawley, 1985) or decreased (Weischer, 1976). Recently, Takeda et al. (1998) demonstrated using an automatic hole-board apparatus that diazepam and chlordiazepoxide

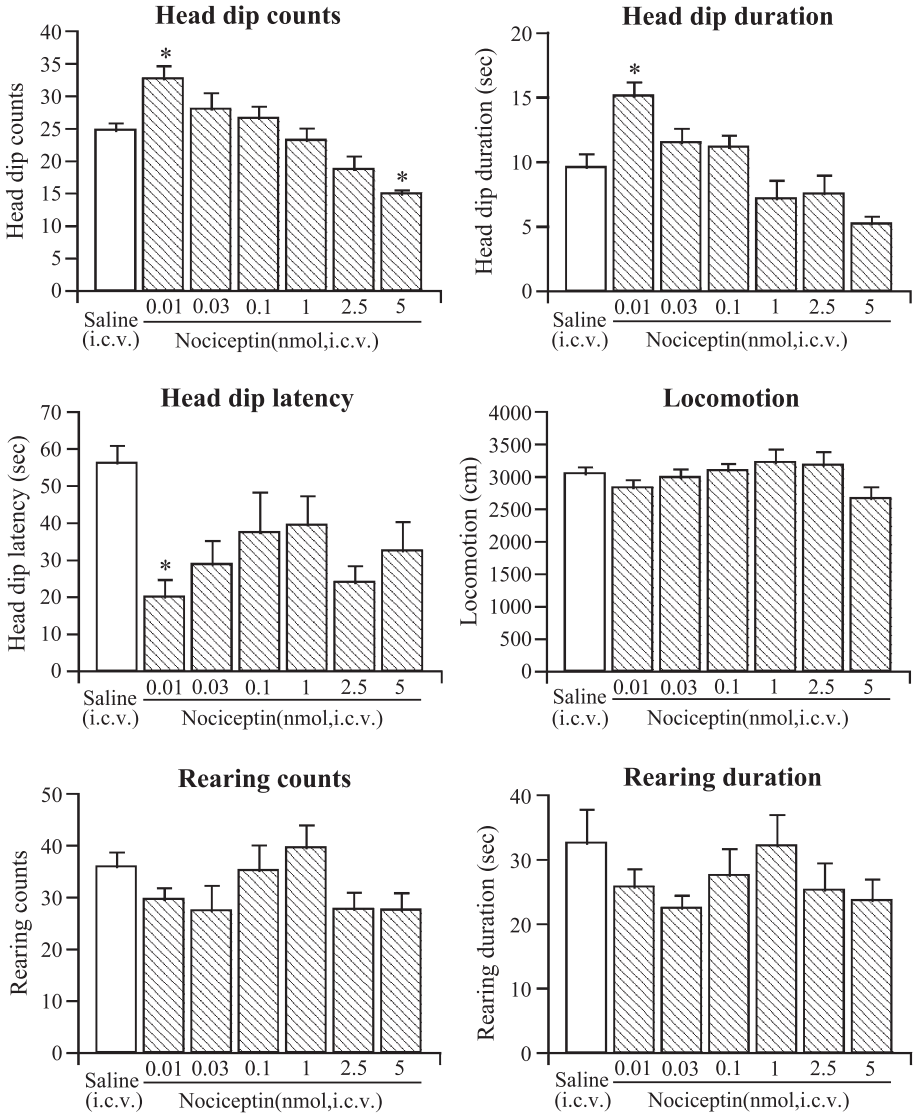

Fig. 1. Effect of nociceptin on exploratory behavior of mice in the hole-board test. Nociceptin (0.01–5 nmol, i.c.v.) or saline was injected 30 min prior to the measurement of exploratory behavior. Each column represents the mean with S.E. of 9–17 mice. *$P<0.05$ vs. saline-treated group.

increase head-dipping behavior, while benzodiazepine receptor inverse agonists, such as methyl-β-carboline-3-carboxylate (β-CCM) and *N*-methyl-β-carboline-3-carboxamide (FG7142), decrease head-dipping behavior. Under these conditions, an anxiolytic state in animals may be reflected by an increase in head-dipping behavior, while an anxiogenic state may be reflected by a decrease in head-dipping behavior.

The primary aim of this study was to examine the effect of nociceptin on emotional properties in mice using the hole-board test. We also investigated the role of 5-HT systems in nociception-induced emotional changes.

2. Methods

2.1. Animals

Experiments were conducted with male ICR 6-week-old mice (Tokyo Laboratory Animals Science, Tokyo, Japan), weighing 30–40 g. They had free access to food and water in an animal room that was maintained at 24 ± 1 °C with a 12-h light–dark cycle. This study was carried out in accordance with the Declaration of Helsinki and the guide for the use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

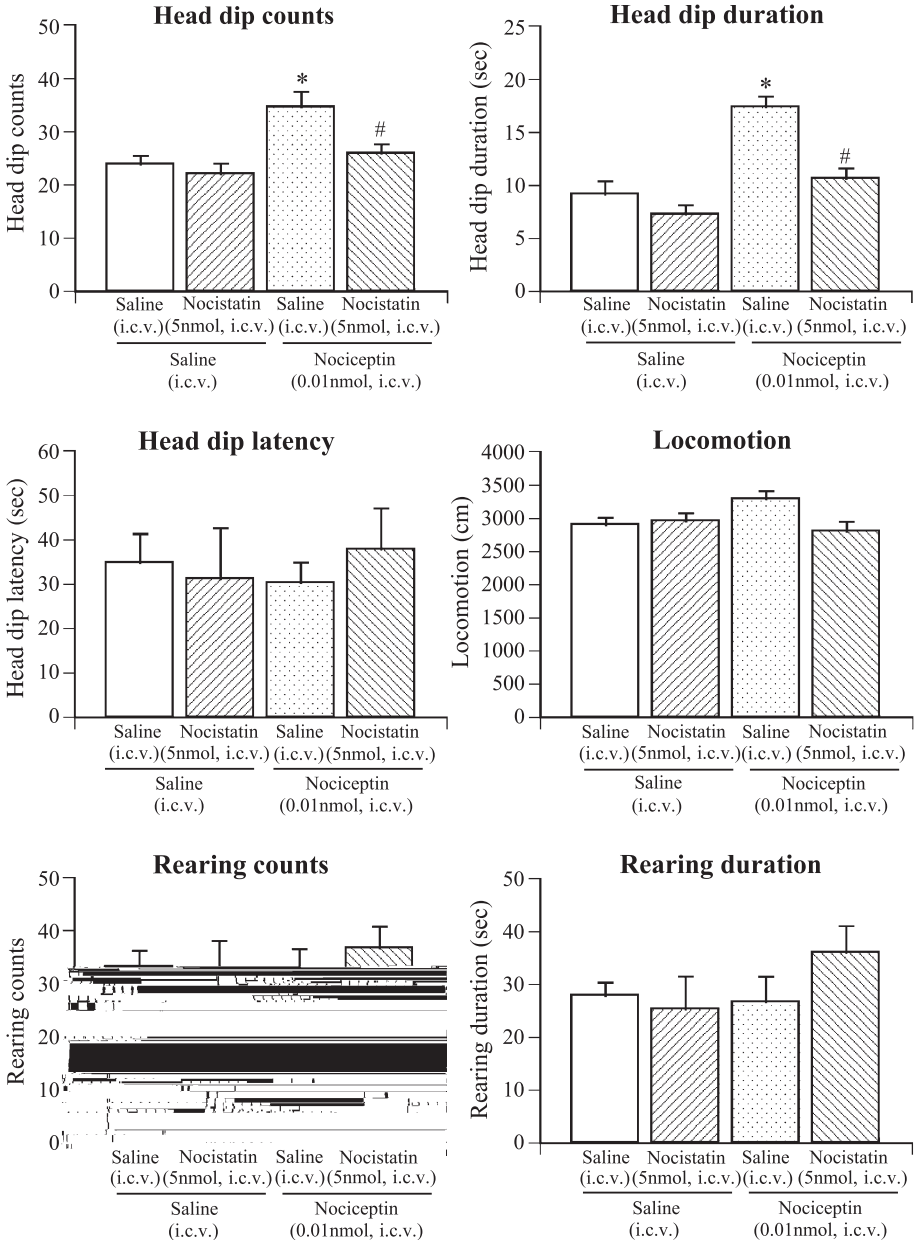

Fig. 2. Effect of nocistatin on the nociceptin (0.01 nmol, i.c.v.)-induced behavior responses of mice in the hole-board test. Nociceptin (0.01 nmol, i.c.v.) was administered 30 min before testing. Nocistatin (5 nmol, i.c.v.) was given just before nociceptin injection. Each column represents the mean with S.E. of 9–12 mice. *$P<0.05$ vs. saline/saline-treated group (open column). $^{\#}P<0.05$ vs. saline/nociceptin-treated group.

2.2. Behavioral studies

The results in the hole-board test were determined automatically as described previously (Takeda et al., 1998; Kamei et al., 2001). The hole-board apparatus was made of a gray wooden box (50 × 50 × 50 cm) with four holes 3 cm in diameter equally spaced in the floor. An infrared beam sensor was installed on the wall to detect the numbers and duration of rearing and head-dipping behaviors, and the latency to the first head-dipping. Other behavioral parameters, such as the locus and distance of movement (total locomotor activity (cm)) of mice, were recorded by an overhead color CCD camera. The heads of the mice were painted yellow and the color CCD camera followed the center of gravity. Data from the CCD camera were collected through a custom-designed interface (CAT-10, Muromachi Kikai, Japan) as a reflection signal. Head-dipping behaviors were double-checked via an infrared beam sensor and an overhead color CCD camera. All of the data were analyzed and stored in a personal computer using analytical software (Comp ACT HBS, Muromachi Kikai, Japan).

For the hole-board experiments, each animal was placed in the center of the hole-board and allowed to freely explore

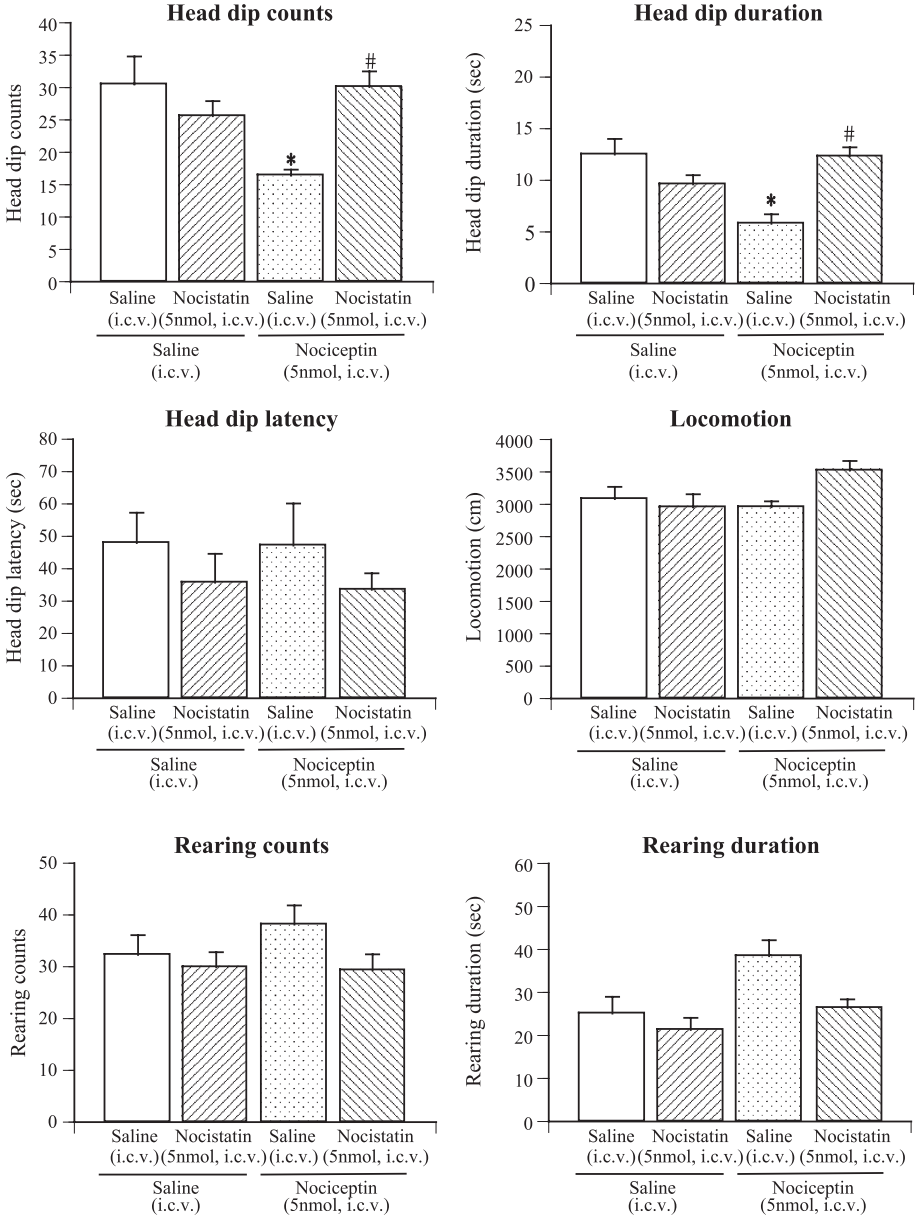

Fig. 3. Effect of nocistatin on the nociceptin (5 nmol, i.c.v.)-induced behavior responses of mice in the hole-board test. Nociceptin (5 nmol, i.c.v.) was administered 30 min before testing. Nocistatin (5 nmol, i.c.v.) was given just before nociceptin injection. Each column represents the mean with S.E. of 9–11 mice. *$P<0.05$ vs. saline/saline-treated group (open column). $^{\#}P<0.05$ vs. saline/nociceptin-treated group (dotted column).

the apparatus for 5 min. Total locomotor activity, numbers and duration of rearing and head-dipping, and the latency to the first head-dipping were recorded automatically. Each mouse was used only once.

2.3. Intracerebroventricular (i.c.v.) injection

One day before the beginning i.c.v. of drug or vehicle injection, mice were anesthetized with ether and a 3-mm double-needle (tip: 27 gauge × 3 mm and base: 22 guage × 5 mm; Natume Seisakusho, Tokyo, Japan) attached to a 25-μl Hamilton microsyringe was unilateral injection site to make a hole. The unilateral injection site was 2 mm from either side of the midline between the anterior roots of the ears. One the day for the i.c.v. injection, the head of the mouse was held against a V-shaped holder, and the drugs were injected into the hole. The injection volume was 5 μl for each mouse. The placement for the injection was confirmed by injection of dye solution after all experiments.

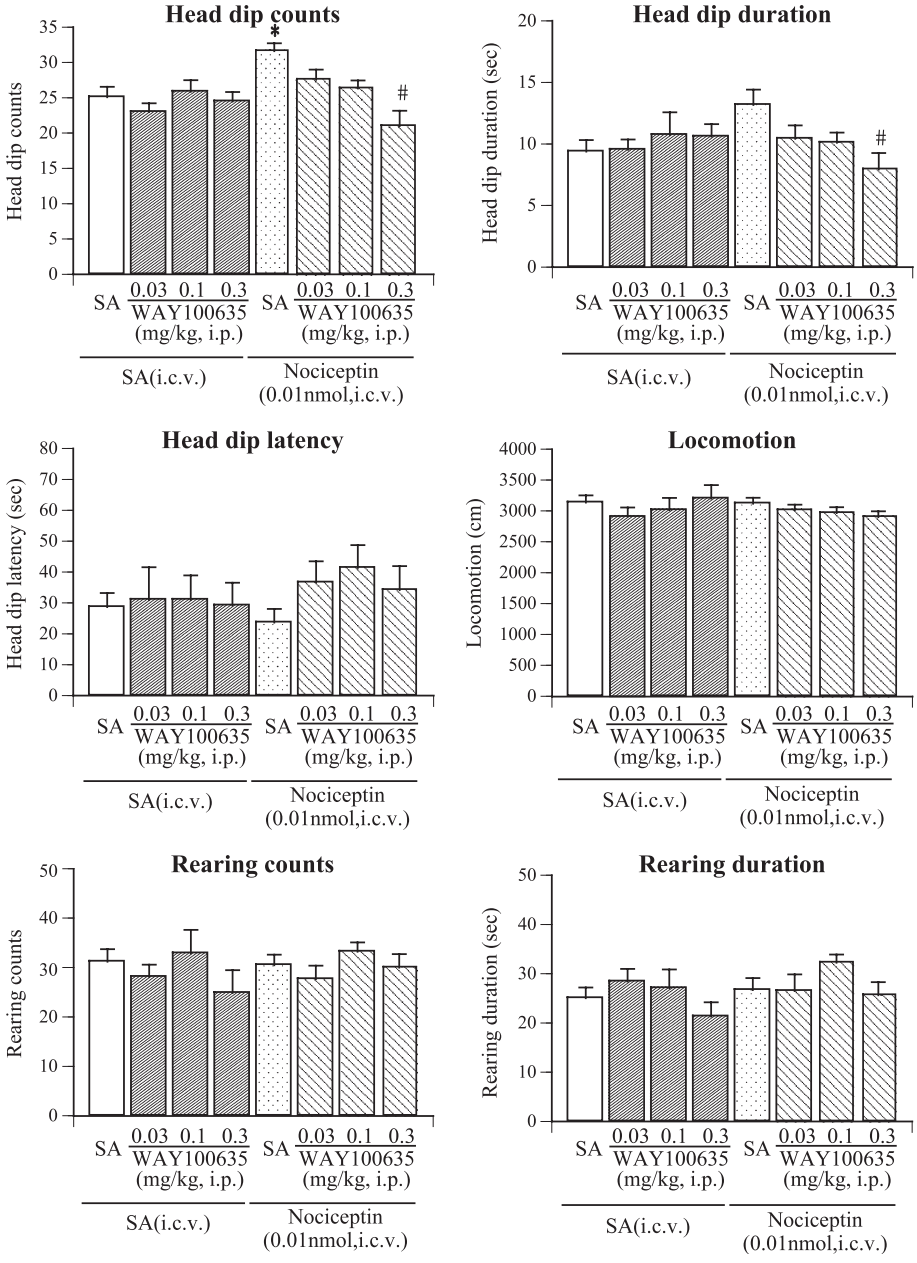

Fig. 4. Effect of WAY100635 on the nociceptin (0.01 nmol, i.c.v.)-induced behavior responses of mice in the hole-board test. Nociceptin (0.01 nmol, i.c.v.) was administered 30 min before testing. WAY100635 (0.03–0.3 mg/kg, i.p.) was given just before nociceptin injection. Each column represents the mean with S.E. of 9–12 mice. *$P<0.05$ vs. saline/saline-treated group (open column). $^{\#}P<0.05$ vs. saline/nociceptin-treated group (dotted column).

2.5. Data analysis

To analyze the behavior and neurochemical data, one-way analysis of variance (ANOVA) followed by Dunnett's test was used for all experiments.

2.6. Drugs

The drugs used in the present study were nociceptin (Peptide Institute, Japan), nocistatin (Peptide Institute, Japan), *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclo-hexanecarboxamide 3HCl (WAY100635, Sigma, St. Louis, MO, USA) and *R*(+)-2-dipropylamino-8-hydroxy-1,2,3,4–tetrahydronaphthalene hydrobromide (8-OH-DPAT: Sigma). All drugs were dissolved in saline.

3. Results

3.1. Effects of nociceptin on exploratory behavior of mice in the hole-board test

Fig. 1 shows the effects of nociceptin on the exploratory behavior of mice in the hole-board test. The lowest dose

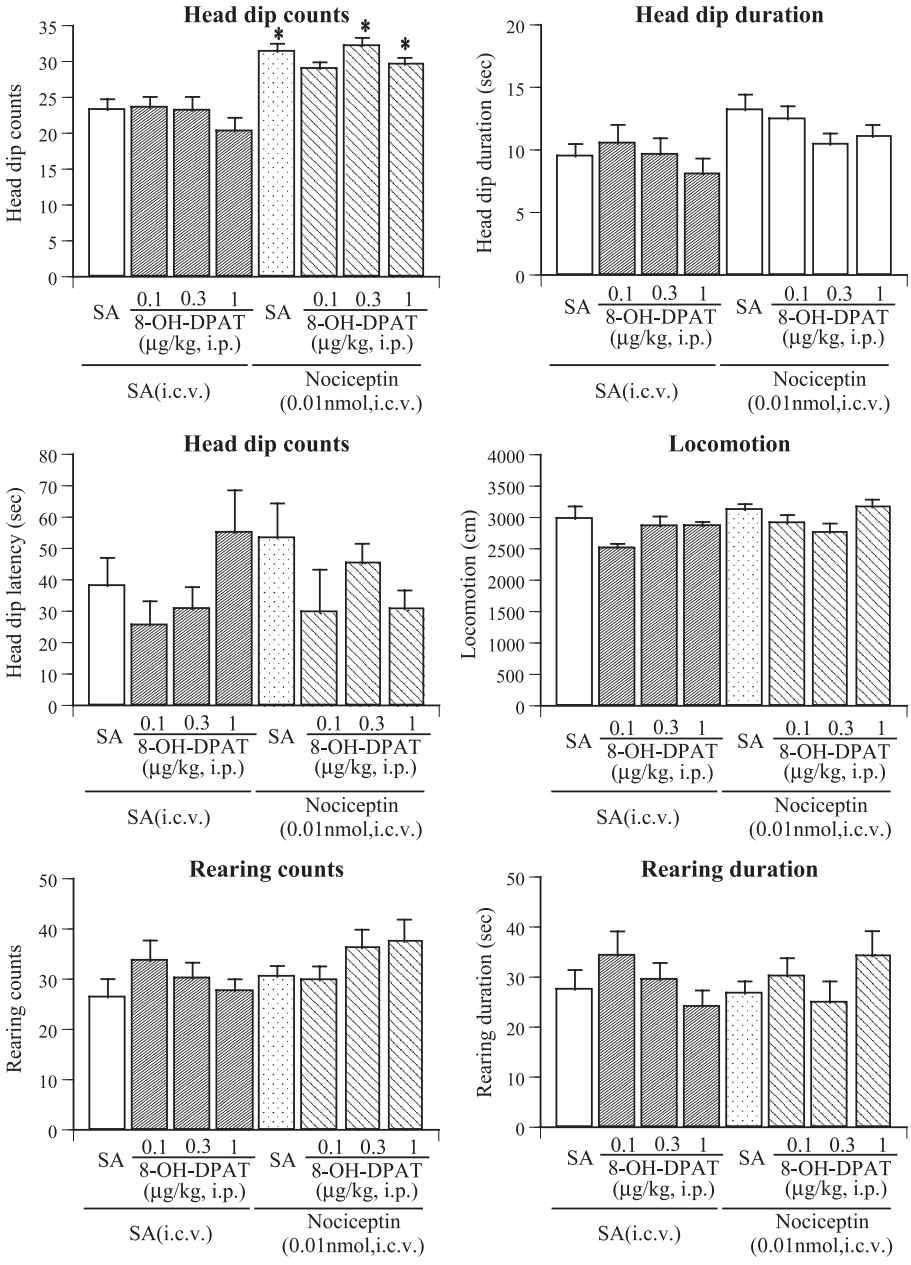

Fig. 6. Effect of 8-OH-DPAT on the nociceptin (0.01 nmol, i.c.v.)-induced behavior responses of mice in the hole-board test. Nociceptin (0.01 nmol, i.c.v.) was administered 30 min before testing. 8-OH-DPAT (0.1–1 μg/kg, i.p.) was given just before nociceptin injection. Each column represents the mean with S.E. of 9–10 mice. *$P<0.05$ vs. saline/saline-treated group (open column).

(0.01 nmol, i.c.v.) of nociceptin significantly increased the counts [$F(6, 73) = 10.2$, $P < 0.05$] and duration of head-dips [$F(6, 73) = 9.5$, $P < 0.05$] and decreased the head-dip latency [$F(6, 73) = 10.2$, $P < 0.05$]. On the other hand, higher doses (0.1–5 nmol, i.c.v.) of nociception dose-dependently reduced the counts and duration of head-dips. Indeed, the highest dose (5 nmol, i.c.v.) of nociceptin significantly reduced the counts of head-dips [$F(6, 73) = 10.2$, $P < 0.05$]. However, nociception, at the doses used in this study, had no effect on locomotor activity, rearing counts or rearing duration in the hole-board test.

3.2. Effects of pretreatment with nocistatin on the nociceptin-induced behavior responses of mice in the hole-board test

The effects of nocistatin, an ORL1 receptor antagonist, on nociceptin-induced behavior responses of mice are shown in Figs. 2 and 3. As shown in Fig. 2, the nociceptin (0.01 nmol)-induced increases in the head-dip counts [$F(3, 28) = 6.7$, $P < 0.05$] and duration [$F(3, 28) = 7.2$, $P < 0.05$] in hole-board test were significantly reduced by pretreatment with nocistatin (5 nmol, i.c.v.). Pretreatment with nocistatin (5 nmol, i.c.v.) also antagonized the nociceptin (5 nmol, i.c.v.)-

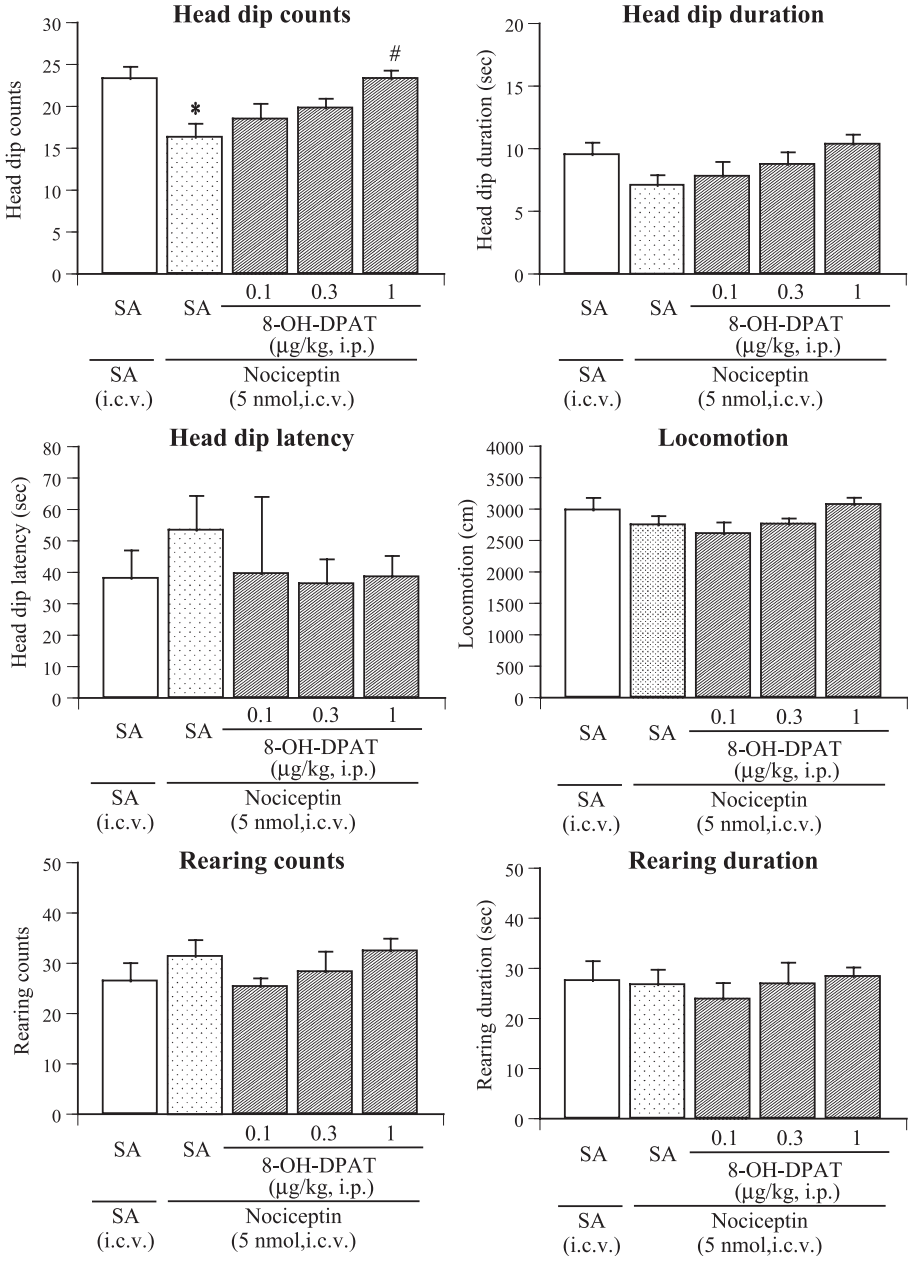

Fig. 7. Effect of 8-OH-DPAT on the nociceptin (5 nmol, i.c.v.)-induced behavior responses of mice in the hole-board test. Nociceptin (5 nmol, i.c.v.) was administered 30 min before testing. 8-OH-DPAT (0.1–1 μg/kg, i.p.) was given just before nociceptin injection. Each column represents the mean with S.E. of 9 to 10 mice. *$P < 0.05$ vs. saline/saline-treated group (open column). $^{\#}P < 0.05$ vs. saline/nociceptin-treated group (dotted column).

induced reduction in head-dip counts [$F(3, 28) = 7.8$, $P < 0.05$] and duration [$F(3, 28) = 7.6$, $P < 0.05$] in the hole-board test. However, nocistatin (5 nmol) by itself had no significant effect on other exploratory behaviors in the hole-board test.

3.3. Effects of pretreatment with WAY100635 on the nociceptin-induced behavior responses of mice in the hole-board test

As shown in Fig. 4, WAY100635 (0.03–0.3 mg/kg, i.p.), a 5-HT_{1A} receptor antagonist, significantly and dose-dependently reduced the low-dose of nociceptin (0.01 nmol, i.c.v.)-induced increase in the head-dip counts [$F(11, 99) = 11.9$, $P < 0.05$] and duration [$F(11, 99) = 4.3$, $P < 0.05$]. However, the reduction in head-dip counts and duration produced by a high dose of nociceptin (5 nmol, i.c.v.) were not affected by pretreatment with WAY100635 (Fig. 5). WAY100635 by itself had no significant effect on other exploratory behaviors in the hole-board test (Fig. 4).

3.4. Effects of pretreatment with 8-OH-DPAT on the nociceptin-induced behavior responses of mice in the hole-board test

Figs. 6 and 7 show the effects of pretreatment with 8-OH-DPAT, a 5-HT_{1A} receptor agonist, on the nociceptin-induced behavior responses of mice in the hole-board test. 8-OH-DPAT (0.1–1 μg/kg, i.p.) significantly and dose-dependently reversed the high-dose nociceptin (5 nmol, i.c.v.)-induced reduction in head-dip counts [$F(11, 94) = 17.4$, $P < 0.05$] (Fig. 7). Furthermore, 8-OH-DPAT (0.1–1 μg/kg, i.p.) dose-dependently, but not significantly reversed the high-dose nociceptin (5 nmol, i.c.v.)-induced reduction in head-dip duration (Fig. 7). However, the increase in head-dip counts produced by low-dose nociceptin (0.01 nmol, i.c.v.) was not significantly affected by pretreatment with 8-OH-DPAT (Fig. 6). 8-OH-DPAT by itself had no significant effect on other exploratory behaviors in the hole-board test.

3.5. Effect of nociceptin on the rate of 5-HT turnover in the mouse amygdala and hippocampus

The effects of nociceptin on the rate of 5-HT turnover in the mouse amygdala and hippocampus are shown in Table 1. The 5-HT ratio (5-HIAA/5-HT) in the hippocampus of nociceptin (0.01 nmol, i.c.v.)-treated mice was significantly greater than that in vehicle-treated mice [$F(2, 27) = 11.0$, $P < 0.05$]. In contrast, a higher dose of nociceptin (5 nmol, i.c.v.) significantly reduced the 5-HT ratio in the amygdala [$F(2, 27) = 6.2$, $P < 0.05$].

4. Discussion

It has been reported that diazepam, a typical benzodiazepine anxiolytic, dose-dependently increased the number and duration of head-dips, whereas the typical anxiogenic drug methyl-βcarboxylate (β-CCM) decreased the number and duration of head-dips and increased the latency to head-dipping (Takeda et al., 1998; Kamei et al., 2001). These results strongly suggest that the changes in head-dipping behavior in the hole-board test may reflect the anxiogenic and/or anxiolytic state of animals. In the present study, a low dose of nociceptin (0.01 nmol, i.c.v.) increased the number and duration of head-dips without significantly changing locomotor activity in mice. On the other hand, high doses of nociceptin (1–5 nmol, i.c.v.) decreased the number and duration of head-dips without significant changing locomotor activity in mice. Thus, it seems likely that nociceptin elicits dose-related anxiolytic and anxiogenic action. Furthermore, both the anxiolytic and anxiogenic effects of nociceptin were antagonized by nocistatin, an ORL1 receptor antagonist. These results suggest that nociceptin elicits dose-related anxiolytic and anxiogenic actions as a result of the activation of ORL1 receptors.

5-HT has been widely assumed to be related to anxiety (e.g., Murphy, 1990). The possible importance of 5-HT_{1A} receptors in anxiety is supported by clinical evidence that 5-HT_{1A} receptor agonists have anxiolytic properties. The

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