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Participation of the nociceptin/orphanin FQ receptor in ethanol-mediated locomotor activation and ethanol intake in preweanling rats

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Abstract

Activation of nociceptin/orphanin FQ (NOP) receptors seems to attenuate ethanol-induced reinforcement in adult rodents. Since early ethanol exposure results in later increased responsiveness to ethanol, it is important to analyze NOP receptor modulation of ethanol-related behaviors during early ontogeny. By measuring NOP involvement in ethanol intake and ethanol-induced locomotor activation, we analyzed the specific participation of NOP receptors on these ethanol-related behaviors in two-week old rats. In each experiment animals were pre-treated with the endogenous ligand for this receptor (nociceptin/orphanin FQ at 0.0, 0.5, 1.0 or 2.0 μ g) or a selective NOP antagonist (J-113397 at 0.0, 0.5, 2.0 or 5.0 mg/kg). Results indicated that activation of the nociceptin receptor system had no effect on ethanol or water intake, while blockade of the NOP receptor has an unspecific effect on consummatory behavior: J-113397 increased ethanol (at a dose of 0.5 mg/kg) and water intake (at 0.5 and 5.0 mg/kg). Ethanol-mediated locomotor stimulation was attenuated by activation of the NOP system (nociceptin at 1.0 and 2.0 μ g). Nociceptin had no effect on basal locomotor activity. Blockade of NOP receptors did not modify ethanol-induced locomotor activation. Contrary to what has been reported for adult rodents, nociceptin failed to suppress intake of ethanol in infants. Attenuation of ethanol-induced stimulation by activation of NOP receptor system suggests an early role of this receptor in this ethanol-related behavior.

Keywords

Ethanol intake; ethanol-induced locomotor activation; infant rat; NOP opioid receptor

1. Introduction

Clinical and preclinical studies agree that prenatal and/or early postnatal ethanol experience predicts later responsiveness to the drug (for review see: [1–6]). This association emphasizes the importance of understanding the neurobiological effects of ethanol early in development, especially considering the growing number of studies showing infant sensitivity to ethanol's

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motivational effects in the rat (reviewed in: [3, 4, 7, 8]). Preweanling rats have proven valuable for assessing these phenomena: infants acquire ethanol-induced first- and second-order place conditioning, readily express ethanol-mediated taste conditioning, are sensitive to ethanol-induced locomotor activation and ethanol-mediated operant responding, and can consume relatively high amounts of the drug without initiation procedures (reviewed in [7]).

It is known that ethanol reinforcement is modulated, at least in part, by distinct components of the endogenous opioid system [9, 10]. Similar to adult rodents, studies from this and other laboratories have shown that, in preweanling rats, the opioid system also regulates ethanol's motivational properties (i.e.: [11–14]). At this time, four major classes of opioid receptors have been identified: mu, delta, kappa, and nociceptin/orphanin FQ peptide (NOP) receptor [15, 16]. These subtypes are widely expressed in brain areas associated with the reinforcing effects of various drugs of abuse including ethanol [10]. Under normal conditions in adult rats, activation of mu and kappa receptors seem to have opposite effects, neurochemically and behaviorally. Specifically, intracerebral administration of mu and kappa receptor agonists, respectively, increase and decrease dopamine release in the ventral tegmental area [17]. Administration of nociceptin/orphanin FQ, the endogenous ligand of NOP receptors, also suppresses the activity of the mesocorticolimbic/dopaminergic system [18].

Recent studies indicated that non-specific opioid antagonists co-administered with ethanol during gestation disrupt future increases in appetitive responding towards ethanol [19–22]. Furthermore, opioid antagonist administration prior to conditioning disrupts appetitive ethanol reinforcement [23]. In newborn and infant rats, the mu and kappa opioid systems modulate ethanol-mediated appetitive reinforcement [14]; selective mu and kappa antagonists inhibit ethanol-induced reinforcement [24]. Ethanol intake by infant rats can also be reduced by non-specific or specific (mu or delta but not kappa) opioid antagonists [12, 22, 25]. Mu opioid receptors are also involved in ethanol-mediated locomotor stimulation. Blockade of mu, but not delta or kappa receptors is effective in attenuating ethanol-induced locomotor activation. Blockade of kappa function facilitates the expression of appetitive ethanol reinforcement in terms of tactile and taste conditioning, and enhances the motor depressing effects of ethanol. Finally, a recent study conducted in this lab indicates the participation of mu, delta and kappa receptors in ethanol-mediated operant responding by infant rats [13]. The only information available regarding the role of the NOP receptor in ethanol reinforcement is from studies conducted in adult rats, and mainly in strains genetically selected for ethanol preference [26].

The NOP receptor is expressed very early in life: it is detected as early as gestational day 12 in the rat and is observed at 16 weeks of gestation in humans [27]. After the first two weeks of postnatal life in the rat, NOP mRNA expression and distribution simulate those observed in the adult brain [27]. Nociceptin/orphanin FQ (from now on called “nociceptin”), the endogenous ligand for NOP receptor, binds with high affinity to the NOP receptor, but apparently does not activate classic opioid receptors (mu, delta, and kappa) [28]. In addition, naloxone, a non-selective opioid antagonist, does not block nociceptin intracellular events [29, 30], confirming that the pharmacological actions of this peptide do not involve classical opioid receptors. Nociceptin has been reported to block opioid-induced supraspinal analgesia, and it has been proposed as a “functional anti-opioid peptide” in the control of brain nociceptive processes [31, 32]. Several studies investigated whether nociceptin behaves as a functional anti-opioid peptide in relation to the motivational properties of ethanol, which are largely dependent upon the opioid system [9, 33]. Studies using genetically selected Marchigian Sardinian alcohol-preferring rats (msP), demonstrated that chronic intracerebroventricular (i.c.v.) nociceptin injections reduced ethanol intake and self-administration, ethanol-induced conditioned place preference [34–36], and environmental conditioning or stress-induced reinstatement of alcohol seeking behavior [36, 37]. The

inhibitory effect of nociceptin on ethanol reinforcement was extended to other rewarding drugs: it reduced morphine withdrawal [38] and CPP induced by morphine, cocaine, and amphetamine [39–41]. Furthermore, Ciccocioppo et al. [35] showed that blockade of NOP receptors did not modify ethanol intake per se, but abolished the effect of an agonist on ethanol intake.

So far, these previous studies indicate that nociceptin controls a variety of ethanol-related behaviors in rats. Currently, to our knowledge, there is no information concerning the modulatory role of NOP receptors on ethanol's motivational effects during early ontogeny. Taking this evidence into account, we decided to test NOP modulatory effects on ethanol intake and ethanol-mediated locomotor activation in infant (2–3 weeks old) rats. Drug consumption and locomotor stimulation were assessed given previous studies from this laboratory indicated that some, but not all opioid receptors, modulate these ethanol-related behaviors in rats of this age [12]. As an initial approach, we decided to employ a selective agonist and antagonist for NOP receptors. Nociceptin was employed as the endogenous ligand and J-113397 as a selective antagonist for the receptor [42]. For each experiment, we used a specific ethanol concentration known to be consumed readily [43], a dose of ethanol effective for exerting locomotor stimulant effects [44] and a wide range of doses of the NOP agonist and antagonist.

2. General methods

2.1 Subjects

Sprague–Dawley infant rats (PDs 13–15) were used across all experiments (Experiment 1a: 85; Experiment 1b: 76; Experiment 2a: 96; Experiment 2b: 93; Experiment 3a: and Experiment 3b: 32 animals). These animals were born and reared in the vivarium at the Center for Development and Behavioral Neuroscience (AAALAC-accredited facility, Binghamton University, Binghamton, NY, USA). Births were examined daily, and the day of birth was considered PD 0. The colony was maintained at 22–24 °C under a 14/10 hr light/dark cycle. The experiments were approved by the Binghamton University Institutional Review Committee for the Use of Animal Subjects and were in compliance with the NIH Guide for the Care and Use of Laboratory Animals [45].

2.2 Drug preparation and administration procedures

The NOP endogenous ligand, nociceptin (TOCRIS, Ellisville, MO), was dissolved in saline (NaCl 0.9% v/v) and injected in a volume of 2 µl/rat. Nociceptin was tested at doses of 0.0, 0.5, 1.0 or 2.0 µg/rat. The drug was administered into the cisterna magna (IC) using a 30-gauge hypodermic needle attached to a length of polyethylene tubing (PE-10). The needle was inserted under visual guidance into the foramen magnum between the occipital bone and the first cervical vertebra [46, 47]. Successful placement of the needle into the target site was confirmed by the appearance of cerebrospinal fluid in the tubing. The solution containing nociceptin was injected in a 5-second pulse with a micrometer syringe (Gilmont Instruments, Barrington, IL) driven by a rotatory microsyringe pump (Kashinsky-Rozboril, Model 5/2000, Binghamton, NY). This volume of infusion does not seem to cause any discomfort or distress and is not excessive for an infant rat, which weighs 35 to 40 g. The NOP antagonist J-113397 [(±)-1-[(3R*,4R*)-1-(Cyclooctylmethyl)-3-(hydroxymethyl)-4-piperidinyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one] (TOCRIS, Ellisville, MO) was dissolved in saline and injected at a dose of 0.0, 0.5, 2.0 or 5.0 mg/kg. The volume of injection was kept at 0.01 ml/g. J-113397 was administered via intraperitoneal (i.p) injection.

2.3 Intake Test

On PD's 14 and 15, infants were separated from their dams, cannulated and placed in pairs in a holding cage (45×20×20 cm). The animals remained in the cage for 2 h. Intraoral cannulation was performed using a procedure described in previous studies [19, 48]. Cannulae were made from 5-cm sections of polyethylene tubing (PE-10, Clay Adams, Parsippany, NJ) by heating one end of the section to form a small flange. A thin wire attached to the non-flanged end of the cannula was placed on the internal surface of the pup's cheek and the wire was then pushed through the oral mucosae until the flanged end of the cannula was positioned over the internal surface of the cheek while the remainder of the cannula exited from the oral cavity. The entire procedure took less than 5 s per pup. The cannulas were later used to infuse the solutions during the intake test. After the two-hour separation, pups were quasi-randomly assigned (with the constraint that no more than one pup of a given sex from the same litter be assigned to the same condition) to the treatments defined by the solution infused: water or 5.0% ethanol v/v (190-proof ethanol, Pharmacology, Brookfield); and the NOP drug administered (Experiment 1a: nociceptin at 0.0, 0.5, 1.0 or 2.0 µg; Experiment 1b: J-113397 at 0.0, 0.5, 2.0 or 5.0 mg/kg). Nociceptin was injected 2 min before the intake test and J-113397 was administered 30 min before this test. Before test, pups' bladders were voided by gentle brushing of the anogenital area. Body weights were then registered. The mean weight of the subjects was calculated and used as a benchmark for the volume of the intraoral infusion of the solution. Each subject's cannula was connected to a length of PE50 tubing that in turn was connected to a 10 ml syringe operated by an automatic infusion pump (KD Scientific, Holliston, MA). The subjects were placed into a Plexiglas container divided into eight sections measuring 9×15 cm each. The bottoms of these containers were lined with absorbent paper and slightly heated (26–27°C). The total volume administered to each subject was equivalent to 5.5% of their body weight and was infused at a constant rate for 15-min. These infusion parameters allow pups to either accept or reject the infused solution. Immediately following the intraoral infusion, pups were disconnected from the tubing and weighed to estimate consumption scores. Ninety minutes later, pups were returned to their dams. On PD15, subjects had the same manipulation as on PD14 and were tested again for water or ethanol intake. The intake test was conducted on two consecutive days because under similar experimental circumstances it has been observed that two consecutive days of testing are sensitive to effects on ethanol intake at this age (for example see [19, 49, 50]). Consumption of ethanol, in terms of grams per kilogram (g/kg) of ethanol ingested during each daily intake test, was calculated according to the following equation: $[(\text{post-test weight} - \text{pre-test weight}) \times (0.05 \times 100)] \times 0.81 / (\text{pre-test weight} \times 1000)$. Water intake was estimated through the percentage of body weight gained during the test: $[(\text{post-test weight} - \text{pre-test weight}) / \text{pre-test weight}] \times 100$.

2.4 Locomotor Activity Assessment

On PD 13, the day before testing, pups were separated from the dam and placed in pairs in holding chambers. One hour later subjects were placed for 8 min in the testing chamber to habituate them to the experimental procedure. Arias and colleagues showed that longer pre-exposure to the testing environment may interfere with the expression of ethanol-induced locomotor activation in preweanling rats [51]. On PD 14, pups were separated from their mothers and kept under similar conditions as the day before. In Experiment 2a, ninety minutes after maternal separation, pups were injected with nociceptin (0.0, 0.5, 1.0 or 2.0 µg). Two minutes later, pups received an intragastric (i.g.) administration of 0.0 or 2.5 g/kg ethanol (volume administered was equivalent to 0.015 ml/g of body weight of a 21% ethanol solution). Pups assigned to the control condition (0 g/kg) received only vehicle (water). Intragastric administrations were performed using a 10-cm length of polyethylene tubing (PE-10) attached to a 1 ml syringe with a 27 G×1/2 needle. This tubing was gently introduced through the mouth and slowly pushed into the stomach. The entire procedure

took less than 20 s per pup. Five minutes after ethanol administration, locomotor activity was assessed for 8 minutes [44]. For Experiment 2b, 1-hr after maternal separation, body weights were individually recorded (± 0.01 g) and pups received an injection of J-113397 (0.0, 0.5, 2.0 or 5.0 mg/Kg). After receiving the injection, pups were again placed in pairs in the holding chamber. Thirty minutes after administration of this NOP antagonist, subjects were administered 0.0 or 2.5 g/Kg ethanol as described before. Five minutes after ethanol administration, locomotor activity was evaluated for 8 min. The testing environment consisted of an open field chamber with Plexiglas walls (42×42×30 cm; VersaMax Animal Activity Monitoring System, Accuscan Instruments, Columbus, OH, USA). In this apparatus, locomotion was detected by interruption of eight pairs of intersecting photocell beams evenly spaced along the walls of the testing environment. This equipment was situated in sound-attenuating chambers (53×58×43 cm) equipped with a light and fan for ventilation and background noise. Consecutive photocell beam interruptions were translated to distance traveled in cm by the VersaMax software. This dependent variable takes into account the path the animal takes, and is an accurate indicator of ambulatory activity. Immediately after the locomotor activity test pups were returned to their home cage.

2.5 Determination of blood ethanol content and corticosterone responsiveness

In order to analyze possible effects of NOP receptor activation on ethanol metabolism or corticosterone (CORT) levels (see Section 3, Results from Experiment 2a), blood ethanol content (BEC), and CORT responsiveness were measured in animals that had the same manipulation as used for the locomotor activity test. In Experiment 3, PD14 infant rats were separated from their mothers and placed in pairs in holding chambers. Ninety minutes later, animals receive an IC administration of nociceptin (0.0, 0.5, 1.0, 2.0 μ g). After receiving the IC injection, pups were administered 0.0 or 2.5 g/Kg ethanol via intubation. Nine minutes after ethanol administration (analog timeline to the middle of locomotor activity test), animals were sacrificed and trunk blood was collected. Samples were collected using a 1.5-ml Eppendorf tube and immediately centrifuged at high speed (10-min at 10,000 rpm; micro-Haematocrit Centrifuge, Hawksley & Sons LTD, Sussex, England). Serum was removed from the samples and stored at -70°C for later analysis. An AM1 Alcohol Analyzer (Analox Instruments, Lunenburg, MA) was used to process BEC. Calculations of BECs are made in terms of conversion rate of ethanol oxidation to acetaldehyde in the presence of ethanol oxidase. The apparatus measures the rate of oxygen required by this process, which is proportional to ethanol concentration, and produces a printout 20 to 30 seconds after the plasma is injected. All BEC values were expressed as milligrams of ethanol per deciliter of body fluid (mg/dl). CORT levels were obtained by means of radioimmunoassay of the plasma samples using a tritium-based kit supplied by MP Biomedicals (Orangeburg, NY), with 100% specificity for rat CORT. The plasma was incubated with CORT and the anti-corticosterone serum. Standards and samples were assayed in pairs, with disintegrations averaged in a minute-by-minute basis using a standard curve performed with each assay. GraphPad Prism 2.0 (GraphPad Software, La Jolla, CA) was employed to input the data and calculate the values obtained in each sample. Values were expressed as ng/ml.

2.6 Experimental design and data analysis

Each experimental group consisted of 9–11 subjects. To prevent the confounding effect of litter, no more than one male and one female per litter were assigned to each particular treatment. Data obtained from intake were analyzed with analysis of variance (ANOVA). Separate ANOVAs were executed to analyze water and ethanol consumption scores. For the locomotion activity test, a preliminary ANOVA was conducted in order to analyze possible effects of NOP activation/blockade of basal motor activity (water control groups); in absence of any significant main effect, water-control groups were collapsed for further

analysis. The loci of significant main effects or two-way interactions were analyzed with Fisher's LSD post hoc comparisons. A rejection criterion of $p < 0.05$ was adopted for all statistical analysis in the present study. Preliminary statistical considerations indicated that across variables and experiments, the factor gender did not exert significant main effect or significantly interact with the remaining factors. Therefore, data were collapsed across sex for all of the subsequent analyses. The lack of sex effects was not unexpected: previous work found that ethanol-mediated intake [20, 49, 50] and ethanol-induced motor activation [12, 44] are similar in male and female preweanling rats of the present age.

3. Results

Experiment 1a

A 4 (nociceptin concentration: 0.0, 0.5, 1.0 or 2.0 μg) $\times$ 2 (postnatal days: 14 and 15) mixed analysis of variance (ANOVA) was employed to process ethanol consumption scores. As can be seen in Figure 1, intake of the drug was significantly affected by the day of evaluation ($F(1, 38) = 9.15$, $p < 0.01$): ethanol consumption significantly decreased on the second day of testing. Administration of nociceptin did not significantly affect consumption of the drug. For water intake profiles, a 4 $\times$ 2 mixed ANOVA indicated no significant effect or interaction of the factors under consideration.

Experiment 1b

A 4 (J-113397 dose: 0.0, 0.5, 2.0 or 5.0 mg/Kg) $\times$ 2 (postnatal days: 14 and 15) mixed analysis of variance (ANOVA) was employed to analyze ethanol consumption. Ethanol intake was significantly affected by the interaction of these two factors ($F(3, 34) = 4.07$, $p < 0.025$). Fisher post-hoc tests indicated that on PD14, infants treated with J-113397 at 0.5 mg/Kg significantly increased ethanol intake compared with saline-injected infants. At PD15 no significant effect could be observed. The 4 $\times$ 2 mixed ANOVA employed to analyze water intake found a significant effect of the NOP antagonist with J-113397 at 0.5 and 5.0 mg/kg significantly increasing water consumption. This effect was independent on the day of evaluation. These results are summarized in Figure 2.

Results of Experiment 1 indicated that ethanol consumption in preweanling rats was differentially affected by NOP receptor blockade or activation. The selective antagonist J-113397 produced an increase in ethanol intake during the first day of testing; this effect was also found for water intake. On the other hand, activation of NOP receptors by its endogenous ligand, nociceptin, produced no evident effect on ethanol or water intake.

Experiment 2a

A preliminary one-way ANOVA was conducted to analyze possible effects of nociceptin on basal locomotor activity. This analysis indicated no significant effect of nociceptin on locomotor activity of water-intubated animals ($p > 0.65$). For further analyses, water-control groups were collapsed across nociceptin treatment. Ethanol-induced locomotor activation was analyzed through a one-way ANOVA which include nociceptin concentrations as the main factor (untreated group, 0.0, 0.5, 1.0, 2.0 μg or water-control). This ANOVA indicated a significant effect of nociceptin ($F(4, 77) = 3.4$, $p < 0.025$): administration of ethanol before testing significantly increased locomotor activity of the animals. In addition, nociceptin at 1 μg was effective in attenuating ethanol's motor stimulating effects, since these animals displayed significantly lower scores of motor activity when compared with ethanol-saline animals and were not significantly different from water-control animals. Nociceptin at 2 μg also attenuated ethanol's stimulant effects: the locomotor activity of these animals was not significantly different from water-control animals but when compared with the ethanol-saline group, only a borderline effect was observed ($p = 0.08$). Finally, animals intubated

with water displayed significantly less locomotor activity than all the other groups, except when compared with ethanol-nociceptin at 1 or 2 μ g. These results are summarized in Figure 3.

Experiment 2b

A preliminary ANOVA was conducted to analyze the effects of J-113397 on basal motor activity. This one-way ANOVA indicated no significant effect of the NOP antagonist on locomotor activity of water-intubated infants ($p > 0.9$). Taking this result into account water-control groups were collapsed across J-113397 doses. Ethanol-induced locomotor activation was analyzed through a one-way ANOVA which included J-113397 doses as a factor (0.0, 0.5, 2.0, 5.0 mg/Kg or water-control). The ANOVA indicated a significant effect of dose of J-113397 ($F(4, 88) = 4.01, p < 0.01$). Animals intubated with ethanol displayed significantly greater locomotor activity than water-control group. The administration of J-113397 did not significantly affect ethanol stimulatory effects. The locomotor activity scores [expressed in distance travelled (cm; mean $\pm$ SE)], for each experimental group was the following: in ethanol-intubated animals, J-113397 at 0.0: 1169.67 ± 179.16 ; at 0.5: 1252.42 ± 209.31 ; at 2.0: 1238.58 ± 240.67 ; at 5.0: 1214.92 ± 234.97 ; in water-intubated animals, J-113397 at 0.0: 718.09 ± 134.64 ; at 0.5: 706.82 ± 184.35 ; at 2.0: 669.55 ± 82.43 ; at 5.0: 725.33 ± 134.48 .

In summary, results from Experiment 2 indicate that NOP receptors do modulate ethanol's motor stimulating effects. Activation of these receptors attenuated ethanol-induced locomotor stimulation with no side effect on basal locomotor activity while blockade of these receptors using a selective antagonist produced no evident change in ethanol-mediated or basal locomotor activation.

Experiment 3

A one-way ANOVA analyzed blood ethanol levels following ethanol administration under different doses of nociceptin. This analysis indicated no significant effect of the factor on BEC ($p > 0.23$). The values of BEC for each group, expressed in mg/dl, were the followings (mean $\pm$ SE expressed in mg/dl): nociceptin at 0.0 μ g: 122.88 ± 9.84 ; at 0.5: 144.17 ± 8.79 ; at 1.0: 150.52 ± 10.42 ; at 2.0: 151.18 ± 19.55 . The two-way ANOVA (ethanol dose $\times$ nociceptin dose) employed to analyze CORT responsiveness indicated that none of the factors or their interaction reached significance (p 's values > 0.7). CORT levels (mean $\pm$ SE, expressed in ng/ml*1000) for each group were the followings: W-NC 0.0: 70.12 ± 9.41 ; W-NC 0.5: 72.57 ± 8.57 ; W-NC 1.0: 87.86 ± 13.28 ; W-NC 2.0: 88 ± 3.68 ; E-NC 0.0: 80 ± 16.32 ; E-NC 0.5: 86 ± 10.1 ; E-NC 1.0: 74.83 ± 9.62 ; E-NC 2.0: 88 ± 19.11 (W: water; NC: nociceptin; E: ethanol).

Data from Experiment 3 indicated that nociceptin, at the doses employed in the current study, did not significantly affect ethanol metabolism or corticosterone responsiveness. In addition, ethanol administration before locomotion evaluation did not significantly modify corticosterone levels.

4. Discussion

According to the present results, we failed to observe an effect of NOP receptor agonist on ethanol intake. Contrary to expectations, activation of NOP receptors by its endogenous ligand did not modify the intake profile of ethanol on any testing day. Nociceptin also failed to affect water consumption. Blockade of these receptors increased both ethanol and water intake. Ethanol-mediated locomotor stimulation in preweanling rats seems specifically to be mediated by NOP receptors: stimulation of these receptors by nociceptin attenuated ethanol-

induced locomotor activation during the early phase of intoxication, while blockade of the NOP receptors had no evident effect on ethanol's stimulatory effects.

In Experiment 1a, nociceptin had no effect on ethanol intake in infant rats. These results were unexpected in view of evidence in genetically selected adult rodents. Previous studies have shown that nociceptin, at concentrations similar to those used here (i.e., 0.5–1.0 µg), were effective in reducing ethanol consumption in Marchigian Sardinian alcohol-preferring (msP) rats, an animal model of high ethanol intake [34]. These results were later replicated using novel agonists for NOP receptors [35]. In contrast, in non-selected Wistar adult rats, nociceptin did not alter ethanol consumption [52]. On the other hand, we did find an effect of NOP blockade on general intake. In Experiment 1b, blockade of NOP receptor by systemic administration of the selective antagonist J-113397 increased consumption of the ethanol, but only during the first day of intake testing. Yet, this effect was unspecific to ethanol, since the NOP antagonist also increased water intake. This effect could be related to an increase in avidity for liquids in general rather than an increase in the reinforcing/rewarding effects of ethanol. It is known that other opioid antagonists may interfere with consummatory behavior in general, an effect observed not only in adults [53] but also in infant rats [12, 19]. Moreover, a recent study indicated that wild type and NOP-KO mice do not differ in positive taste reactions to sucrose, but did differ in aversive reactions to various quinine concentrations [54], a solution that would share the bitter taste quality of ethanol [55–56]. Regarding NOP blockade in adult rats, a study employing msP rats indicated that, although a pretreatment with selective NOP antagonists was effective in abolishing the effects of the agonist, the antagonists per se did not modify ethanol intake [35], even when the animals received the antagonists for as long as 8 days. Differences between effects in the present study and those using msP rats could be due to differences in the rat strain and/or level of exposure to the drug. Interestingly, it has been shown that basal levels of nociceptin are markedly different among mouse lines that vary in their ethanol preference and are influenced by repeated ethanol administrations [57, 58]. Concerning a more general effect not related to ethanol, it has been well characterized that there is a renal excretory response (water diuresis) evoked by nociceptin i.c.v. administration to conscious and anesthetized rats [59, 60]. So it is possible that this diuretic effect of nociceptin may influence fluid intake. Nonetheless, our results indicate no significant effect of the activation of NOP receptors on ethanol or water intake. The consummatory behavior was affected when NOP receptors were blockade. In this sense, in a recent study conducted by Wainford and Kapusta [61], Sprague-Dawley rats maintained on a normal salt intake regimen were i.c.v. infused with the selective NOP receptor antagonist UFP-101. These investigators found that NOP blockade failed to produce a change in any renal excretory parameter in normotensive rats [61]. This suggests that a renal effect mediating the observed change in fluid intake in the current study is unlikely.

Data from Experiment 2 indicate that NOP receptors modulate ethanol-mediated locomotor stimulation. In this case, the stimulatory effect of ethanol during the initial state of acute intoxication was attenuated by exogenous activation of NOP receptors (Experiment 2a), but unaffected by blockade of these receptors (Experiment 2b). It is important to note that the effects observed were specific to ethanol-related behaviors and not to a general effect of nociceptin on motor activity. Previous work conducted by Narayanan [62] did, however, find that administration of high doses of nociceptin (30 µg) suppressed motor activity in adult rats, unlike in the current study. This effect seems to be specifically mediated by NOP receptors, since pretreatment with J-113397 (NOP antagonist) attenuated this suppressing effect of nociceptin [62]. This disparity might be explained by the fact that the concentration of the ligand used in the present study was 30-fold lower than used by Narayanan [62]. The lower dose used here did not itself modify basal locomotor activity of the animals. Even though nociceptin suppressive effects were not evident under basal conditions (i.e., water

intubated animals), it is possible that any locomotor depressing effect was additive to the sedative effects of the dose of ethanol employed. In other words while neither the ethanol dose nor the NOP antagonist had a motor depressing effect on their own their cumulative effect depressed locomotor activity. While more research into the possible cumulative effects of ethanol and NOP agonist is needed it seems unlikely to us that this is the case. Previous work in this laboratory has shown that when using a relatively high ethanol dose (2.5 g/kg) pups display locomotor activation soon after administration of the drug (a time point that corresponds with the time of evaluation in the present study). When blood ethanol concentrations reach peak values, ethanol suppresses motor activity [44] an effect evident after 15 or 30 min post ethanol administration.

One question is what mechanism might be responsible for the effects of NOP on ethanol motor activation? A considerable number of studies have shown that ethanol-induced locomotor stimulation is mediated by the dopaminergic system in adult rodents [63–65]. Systemic or local (in nucleus accumbens or ventral tegmental area, VTA) ethanol administration induces dopamine release in the nucleus accumbens [64, 66]. In addition, ethanol-mediated locomotor activation in adult rodents also can be modulated by dopaminergic drugs such as D1- or D2-receptor antagonists [67–69]. More importantly, the same mechanism is shared for ethanol stimulating effect in preweanling rats [70]. On the other hand, it also has been shown that nociceptin can modulate mesolimbic dopamine release [18, 71–73]. Therefore it is plausible that the mechanism that underlies the present effect (i.e., attenuation of ethanol-mediated locomotor stimulation due to administration of nociceptin) could be explained by the suppressive effects of nociceptin on ethanol-mediated mesolimbic dopamine release.

There could also be another explanation for the mechanism underlying the nociceptin-induced attenuation of the ethanol motor stimulatory effect. In adult rats novelty and stress modulate the locomotor stimulating effects of a variety of drugs [74]. Exposure to a novel environment or to an acute stressor induces activation of the hypothalamus–pituitary–adrenal axis [75, 76]. Arias [77] discovered a synergism between stress and ethanol-induced stimulation in preweanling rats. The interaction between stress (induced by social isolation) and ethanol seems to be mediated by the corticosterone release factor (CRF), since blockade of CRF-1 receptors cancelled the effect of ethanol in isolated pups [77]. At the same time, activation of NOP receptors induces anxiolytic-like and anti-stress effects across multiple species (reviewed in [78]). In addition, nociceptin was shown to act as a functional CRF antagonist and to reverse behavioral effects of stress including anorexia [79, 80]. Therefore, it is possible that nociceptin produced an attenuation of ethanol stimulatory effects via action on CRF. On the other hand, several studies suggested that administration of exogenous nociceptin by i.c.v. injection activates basal HPA axis activity and enhances CORT response to mild novel environment stress [81–83]. Experiment 3 was conducted to analyze possible differences in CORT responsiveness after ethanol and nociceptin treatment. Results indicated that ethanol did not alter CORT levels when compared with water intubated animals. A recent study indicated that ethanol-induced increase in corticosterone is more evident later in development [84]. In that study, similar plasma corticosterone levels from P16 rats were found even after a lower or higher ethanol challenge (1.5 and 4.5 g/kg, respectively) and even when samples were collected after 160-min post challenge [84]. Moreover, nociceptin administration did not seem to alter CORT levels. This negative result could be due in part to the absence of ethanol effects on CORT responsiveness. In addition, in the present study blood samples were obtained 9 min after nociceptin injection; whereas in the studies that found differences in CORT levels after nociceptin treatment, samples were collected 30 or 60 min after i.c.v. injection. Even though we cannot discard the hypothesis of nociceptin effects on the CRF system, the present results cannot support it in this specific case.

In summary, this set of studies verified that: activation of NOP receptors has no evident effect on ethanol intake; contrary to previous observations in adult rodents, blockade of NOP receptors increased ethanol and water intake. This effect could be driven by an effect on consummatory behavior in general. The second set of experiments indicated that activation of NOP receptors attenuated ethanol-induced locomotor activation, while blockade of these receptors produced no evident effect. Nociceptin might be attenuating ethanol-mediated stimulation by its suppressive effects on dopamine release and also by its antagonist actions on the CRF receptors. Motor activation induced by psychoactive drugs has been long considered an index, albeit indirect, of positive, rewarding central effects [85, 86]. The fact that nociceptin inhibits this behavioral effect, that is indicative of ethanol's rewarding properties, suggests that NOP receptors may be a promising target for pharmacological manipulation to regulate ethanol's rewarding/motivational effects and also suggests that NOP receptor agonists may have potential as a medication for the treatment of alcohol abuse. Since this is the first study to analyze the participation of NOP receptors in ethanol-related behaviors in preweanling rats, further studies will help to understand how this opioid receptor modulates ethanol's motivational effects during early ontogeny.

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HIGHLIGHTS

- Infant rats are sensitive to ethanol's reinforcing properties.
- Activation of nociceptin/orphanin FQ (NOP) receptors failed to suppress intake of ethanol but it did attenuate ethanol's locomotor stimulatory effects in infant rats.
- ◆ Blockade of NOP receptors affected consummatory behavior in general, increasing ethanol and water intake.

Figure 1. Consumption of 5.0% ethanol (expressed in g/kg, panel A) or water (expressed in %BWG, panel B) during PD14 and PD15 as a function of nociceptin treatment (0.0, 0.5, 1.0 or 2.0 μg /rat). Vertical lines represent standard error of the mean.

Figure 2. Consumption of 5.0% ethanol (expressed in g/kg, panel A) or water (expressed in %BWG, panel B) during PD14 and PD15 as a function of J-113397 treatment (0.0, 0.5, 2.0 or 5.0 mg/kg). Vertical lines represent standard error of the mean.

Figure 3. Locomotor activity in PD14 rats as a function of ethanol (0.0 or 2.5 g/kg) and nociceptin (0.0, 0.5, 1.0 or 2.0 µg) treatment. Vertical lines represent standard error of the mean.