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The response to naltrexone in ethanol-drinking rats depends on early environmental experiences

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ABSTRACT

The opioid receptor antagonist naltrexone is currently used in the treatment of alcohol addiction. However, substantial individual differences have been reported for the efficacy of naltrexone. Genetic factors are known to contribute to these differences; however, little is known about the impact of early environmental influences. Based on previous findings that have suggested a link between ethanol, endogenous opioids and the early environment, it was hypothesised that early environmental factors affect naltrexone efficacy later in life. A population of Wistar rats was subjected to three different rearing conditions where the pups experienced a daily separation from the dam, for either 15 min or 360 min, or were just briefly handled. On postnatal day 26, the rats were given intermittent access to ethanol (5% and 20%) and water for six weeks before naltrexone (0.3 mg/kg or 3.0 mg/kg) or saline treatment using a randomised injection schedule with a one-week washout period between injections. Naltrexone reduced ethanol consumption, but there was high variability in the efficacy. In addition, there was an association between the rearing condition and the effectiveness of naltrexone. Naltrexone reduced ethanol intake in rats experiencing postnatal conditions that contrasted normal wildlife conditions, *i.e.*, prolonged absence or continuous presence of the dam, and naltrexone had no effect on the total ethanol consumption in rats reared under naturalistic conditions, *i.e.*, short absences of the dam. These rats reduced their intake of 5% ethanol but increased their preference for 20% ethanol. We conclude that rats with a history of early adversity responded well to naltrexone treatment, whereas rats reared in a social context similar to that found in nature did not benefit from treatment. The present study highlights the importance of not only considering genetics but also environmental factors when identifying individual responses to naltrexone.

1. Introduction

Alcohol use disorders (AUD) are a major health problem with a great need for individualised treatment strategies. Although pharmacotherapy is available today, it is evident that not all patients benefit from general treatment schedules (Spanagel and Kiefer, 2008). To achieve better pharmacotherapy of AUD, knowledge about how each individual responds to ethanol and drugs used for treatment is essential. Ethanol interferes with the function of a number of central transmitter systems, including acetylcholine, glutamate, GABA, monoamines and endogenous opioids (Soderpalm et al., 2009; Vengeliene et al., 2008). Individual differences in any of these networks may contribute to differences in the sensitivity to pharmacological agents and therapeutic outcome.

Opioid networks have attracted interest with regard to the aetiology of AUD and as a target for pharmacological therapy (Modesto-Lowe and Fritz, 2005; Nylander and Silberring, 1998; Oswald and Wand, 2004). Ethanol activates central opioids, although the exact mechanism has not been elucidated, and endogenous opioids have been implicated in addiction processes and in the neurobiological basis for AUD vulnerability (Barson et al., 2009; Gianoulakis, 2004; Trigo et al., 2010; Van Ree et al., 2000). The mu-opioid peptide receptor (MOPR) has been implicated in the actions of ethanol because genetic deletion or antagonism of the MOPR attenuates ethanol-induced mesolimbic dopamine release (Benjamin et al., 1993; Job et al., 2007). Opioid receptor antagonists reduce ethanol consumption in animal and human studies (O'Malley, 1996; Ulm et al., 1995; Volpicelli et al., 1995), and the MOPR receptor antagonist, naltrexone, is an FDA-approved medication for AUD treatment. These studies suggest that opioid antagonists act as anti-reward drugs; when they are administered prior to ethanol intake, the ethanol-induced reward is absent, which leads to a decrease in ethanol intake.

However, an increasing number of reports describe large individual variation in the effects of naltrexone in AUD patients (Kiefer et al., 2008; Spanagel and Kiefer, 2008), and the underlying mechanism has not been elucidated. Pharmacogenetic studies have revealed that genetic factors contribute to individual differences in the ability of naltrexone to reduce ethanol intake (Monterosso et al., 2001; Ray et al., 2010; Rubio et al.,

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2005). For example, several reports have shown that MOPR polymorphisms affect the response to naltrexone (Mague and Blendy, 2010; Oslin et al., 2003). Little is known about the impact of environmental factors on the effects of opioid antagonists. Environmental experiences, especially early in life, may cause long-term changes in neuronal functioning and are known to affect the propensity for AUD (De Bellis, 2002; Langeland et al., 2004). Endogenous opioids represent putative targets for environmentally induced effects. Early in life, brain opioids play an important role in neuronal development and social behaviour (Nelson and Panksepp, 1998). Furthermore, the opioid networks develop and mature postnatally (Loughlin et al., 1985; Petrillo et al., 1987; Spain et al., 1985), and early experiences may evoke long-lasting changes in opioid function. To study the mediators and consequences of early environmental influence, maternal separation (MS) is commonly used. Maternal separation procedures, including the separation of the rat pups from the dam for short or prolonged periods during the first postnatal weeks, are used to assess the consequences of different environmental conditions (Holmes et al., 2005; Ladd et al., 2000; Pryce and Feldon, 2003). Previous studies using MS have shown that endogenous opioids are affected by early environmental factors as evidenced by long-lasting changes in the opioid networks (Gustafsson et al., 2008; Ploj et al., 2003) and an altered pain threshold (Kehoe and Blass, 1986; Pieretti et al., 1991). In addition, neurobiological alterations, caused by early environmental factors, affect the sensitivity to drugs that act on the opioid system. Long-term voluntary ethanol consumption can elicit different effects on opioid peptides and receptors, which is dependent on previous experiences, with enhanced ethanol-induced responses in rats exposed to prolonged MS (Gustafsson et al., 2007; Ploj et al., 2003). Furthermore, rats subjected to prolonged MS had an altered sensitivity to morphine compared to non-handled rats (Kalinichev et al., 2001a; Kehoe and Blass, 1986). Finally, when compared to non-handled rats, MS enhanced the effects of naltrexone on sucrose responses in male Long-Evans rats (Michaels and Holtzman, 2007).

Taken together, these previous studies suggest a link between ethanol, endogenous opioids and early environmental factors. However, it is not known whether the environment early in life also affects the efficacy of naltrexone in reducing ethanol consumption. Therefore, the present study was designed to test whether different environmental experiences early in life result in different responses to naltrexone in ethanol-drinking animals. If this hypothesis is true, environmental factors may contribute to the individual differences seen in treatment outcomes observed in the clinic. A population of Wistar rats with different rearing conditions was given free access to ethanol and water three days a week. After six weeks of drinking, naltrexone was administered prior to a 24 h session with free ethanol access during which its effects on ethanol consumption were assessed. Whether naltrexone differentially affected the consumption of low (5%) and high (20%) ethanol concentrations was also examined. The results provide novel evidence of the different effects of naltrexone and their dependence on rearing conditions.

2. Materials and methods

2.1. Animals

Time-mated outbred Wistar rats from Scanbur BK AB, Sweden, arrived at the animal facility on gestational days 15–16. Animals were then housed singly in standard cages (59×38×20 cm) with wood chip bedding as nesting material and were fed pellet food (R36 Labfor; Lactamin, Vadstena, Sweden) and water *ad libitum*. All animals were housed in temperature (21±1 °C) and humidity controlled (49±5%) animal cabinets in a room on a 12-h light–dark cycle with lights on at 08:00. All animal experiments were performed following an approved protocol in accordance with the Uppsala animal ethical committee and the Swedish Animal Protection Legislation.

2.2. Maternal separation

At birth, all pups from 15 time-mated Wistar rats were sexed, and the litters were arranged to minimise the use of biological littermates in the same experimental groups and culled into 10 pups (5–6 males, 4–5 females) per litter. The litters were separated from the dam for 0 min (MS0; $n=5$ litters), 15 min (MS15; $n=5$ litters) or 360 min (MS360; $n=5$ litters) daily from postnatal day (PND) 1–20. The separation procedure started daily at 10:15 and was performed during the light period. The separation was initiated by removing the dam from the nest to a macrolon cage (26×20×14 cm) containing wood chip bedding material, followed by removing the litter into a separate macrolon cage (26×20×14 cm) containing wood chip bedding material. In the MS15 and MS360 groups, the litters were moved to a heating cabinet (30±2 °C) in an adjacent room during the separation period. The dams in the MS360 groups were returned to their home cages during the separation procedure but were removed prior to the return of the litters. In the MS15 groups, the dams were kept in another cage during the separation, and the litters were returned to the home cages before the dams. In the MS0 groups, the litters were handled similarly to the MS15 and MS360 litters but then returned immediately to the home cage and were not separated from their mother more than 45 s. The rats were weaned on PND 21, and the 12-hr light–dark cycle was adjusted to lights off at 14:00. Thereafter, the male rats were housed three per cage with wood chip bedding material and a wooden house. The cages were changed once a week, and water access was given *ad libitum*. Thirty-four male rats, MS0 ($n=12$), MS15 ($n=11$) and MS360 ($n=11$), were used in the present study. The same person performed all separation sessions and care giving of the rats, and gloves were used during all animal contact. The rats were housed in the same room for the duration of the experiment.

2.3. Voluntary ethanol consumption

On PND 26, the male rats were singly housed in macrolon cages (42×26×18 cm) with wood chip bedding material and a wooden house in temperature (21±1 °C) and humidity controlled (49±5%) animal cabinets. Care was taken to place rats from the three experimental groups (MS0, MS15 and MS360) appropriately to avoid differences due to cabinet housing, and each cage remained in the same place during the length of the experiment, including the ethanol drinking and naltrexone injection periods. The animals were intermittently exposed to ethanol; a procedure that results in higher ethanol intake in rats compared to continuous access models (Simms et al., 2008; Sinclair and Senter, 1967; Wise, 1973). A modified version, previously described (Daoura et al., 2011), was used to examine the consumption of different concentrations of ethanol. At the beginning of the dark cycle, all rats were given free access to three bottles, 5% and 20% ethanol (v/v using 95% ethanol diluted in tap water) and tap water. Water was available at all times. The positions of the bottles (250-mL plastic bottles with ball-valve nipples obtained from Scanbur BK AB, Sollentuna, Sweden) were changed for each session to avoid place preference. Ethanol solutions were changed for each session, and water was changed every day. Body weights were measured before every session.

The rats had free access to ethanol three days a week (Monday, Wednesday, Friday) for nine weeks. During the 6th week of ethanol access, the animals were familiarised to the procedure used for measurement of naltrexone-induced effects. The ethanol consumption was measured at 30 min, 2 h and 24 h after the beginning of ethanol access.

2.4. Naltrexone treatment

During the 7th week of ethanol access, the naltrexone/saline injections started. The injections were performed once a week, on

Mondays over a three-week period. Each rat received three injections, saline and two doses of naltrexone, respectively, with a one-week washout period between injections, according to a randomised injection schedule. Naltrexone (Sigma-Aldrich, Schnelldorf, Germany) was dissolved in saline and administered subcutaneously in one of two dosages: 0.3 mg/kg or 3.0 mg/kg. The injection volume was 1 ml/kg, and the dosages were chosen based on personal experience and previous reports (Mhatre and Holloway, 2003; Williams and Broadbridge, 2009). The drug solutions were prepared immediately before the injections, which were given 30 min prior to the beginning of the dark cycle and ethanol access. The ethanol consumption was measured 30 min, 2 h and 24 h after ethanol access, *i.e.*, 1 h, 2.5 h and 24.5 h after the saline/naltrexone injections. Ethanol intake was also measured on Wednesdays and Fridays to assure that the duration of the washout period was sufficient, *i.e.*, that the effects of naltrexone did not persist.

2.5. Statistical analysis

Using the Shapiro–Wilk test, the ethanol and water intake were not part of a normal distribution. Therefore, nonparametric tests were used for the statistical analysis. The Friedman test followed by the Wilcoxon matched pairs test was used when comparing fluid intake parameters after naltrexone or saline administration. The difference between ethanol consumption after naltrexone and saline injections was calculated in each rat and the naltrexone-induced change was then compared between groups with the Kruskal Wallis test followed by the Mann Whitney *U*-test. Differences were considered statistically significant at $p \leq 0.05$. Statistical analyses were performed using Statistica 8.0 (StatSoft Inc., Tulsa, OK).

3. Results

3.1. Ethanol consumption prior to naltrexone treatment

During the first five weeks of ethanol consumption, the rats established a stable ethanol intake but there were no major differences in the ethanol intake between the MS0, MS15 and MS360 rats. Detailed information on the voluntary ethanol intake during these weeks is described elsewhere (Daoura et al., 2011). The week prior to naltrexone treatment, the weekly intake was 5.9 ± 1.6 g/kg/24 h in the MS0 rats, 5.0 ± 1.0 g/kg/24 h in MS15 rats and 5.2 ± 1.3 g/kg/24 h in the MS360 rats.

3.2. The effects of naltrexone treatment on total ethanol consumption

The analysis of the naltrexone effects in the entire population of Wistar rats revealed decreases in ethanol intake after the naltrexone injection, which were borderline significant in the Friedman test at 30 min [$\chi^2 = 5.9$, $p = 0.053$] and statistically significant at 2 h [$\chi^2 = 10.3$, $p = 0.006$] after ethanol access, *i.e.*, 1 h and 2.5 h, respectively, after the injection. A decrease in ethanol intake was observed after both 0.3 and 3.0 mg/kg naltrexone compared to saline injections. The results from the Wilcoxon test are shown in Fig. 1A and B.

A decrease in total ethanol consumption was observed after naltrexone compared to saline injections in MS0 and MS360 rats, whereas naltrexone treatment had no effect in the MS15 group. In the MS0 rats, a change in ethanol consumption was observed at 30 min [$\chi^2 = 6.5$, $p = 0.038$] and at 2 h [$\chi^2 = 8.6$, $p = 0.013$] after ethanol access. The ethanol consumption was decreased during the first 30 min of ethanol intake after treatment with both 0.3 and 3.0 mg/kg naltrexone compared to saline, Fig. 1A. Additionally, 2 h after ethanol access, the ethanol consumption was reduced after both naltrexone dosages, Fig. 1B.

In the MS360 rats, a difference in ethanol consumption was observed at 30 min [$\chi^2 = 6.7$, $p = 0.035$] and at 2 h [$\chi^2 = 10.4$, $p = 0.006$] after ethanol access. The low dose of naltrexone did not induce any changes in ethanol consumption during the first 30 min of ethanol intake compared to saline, but a decreased intake was seen after 3.0 mg/kg naltrexone, as shown in Fig. 1A. These results were also dose-dependent; the decrease was more pronounced after 3.0 mg/kg than 0.3 mg/kg. Two hours after access to ethanol, both naltrexone doses caused reduced ethanol consumption compared to saline (Fig. 1B). A trend in lower ethanol consumption was also observed with 3.0 mg/kg compared to 0.3 mg/kg naltrexone treatment [$Z = 1.8$, $p = 0.075$].

Twenty-four hours after ethanol access, no differences in total ethanol consumption were observed between saline and naltrexone treated rats (Fig. 1C). No differences were observed in ethanol consumption during the washout period between injections, demonstrating that the effects of naltrexone did not persist into the next treatment period (data not shown).

3.3. The naltrexone-induced decreases in ethanol consumption were dependent on the rearing conditions

The differences between ethanol consumption after naltrexone and saline injections were calculated in each rat to be able to statistically compare the naltrexone-induced effects between groups (Table 1). The Kruskal Wallis test confirmed that the naltrexone-induced effects on ethanol consumption 30 min and 2 h after treatment was different between the MS0, MS15 and MS360 groups, after both 0.3 mg/kg [$H = 10.8$, $p = 0.005$ and $H = 10.2$, $p = 0.006$, respectively] and 3.0 mg/kg [$H = 8.8$, $p = 0.012$ and $H = 7.2$, $p = 0.028$, respectively]. The reduction in ethanol consumption after naltrexone in the MS0 rats and the MS360 rats was statistically significant as compared with MS15 rats that had no effect of naltrexone (Table 1). The only significant difference between the MS0 and MS360 groups was found at 30 min after ethanol exposure; the MS0, but not the MS360, group had reduced ethanol consumption after 0.3 mg/kg naltrexone.

3.4. Naltrexone differentially affects the consumption of 5% and 20% ethanol

The animals had free choice between water, 5% and 20% ethanol. This paradigm examined whether naltrexone affects the intake of these ethanol concentrations similarly or changes the preference for either ethanol concentration. The results revealed that all animals, independent of rearing condition, reduced their intake of 5% ethanol at 30 min and 2 h after ethanol access (Fig. 2). In the MS0 group, a difference in 5% ethanol consumption was observed with the Friedman test at 30 min [$\chi^2 = 10.5$, $p = 0.005$] and at 2 h [$\chi^2 = 6.5$, $p = 0.039$] after ethanol access. The Wilcoxon test revealed decreased intake of 5% ethanol during the first 30 min at both low and high naltrexone doses when compared to saline injections (Fig. 2A).

In the MS15 group, a decrease in 5% ethanol consumption was observed 2 h [$\chi^2 = 9.5$, $p = 0.009$] after ethanol access (Fig. 2B). When compared to saline, reduced ethanol consumption was observed after 3.0 mg/kg but not after 0.3 mg/kg naltrexone treatment. In addition, there was a decrease in ethanol consumption after 3.0 mg/kg naltrexone treatment when compared to 0.3 mg/kg naltrexone.

In the MS360 group, a decrease in 5% ethanol consumption was observed at 2 h [$\chi^2 = 10.4$, $p = 0.006$] after ethanol access (Fig. 2B). Both 0.3 mg/kg and 3.0 mg/kg naltrexone reduced the ethanol consumption. In addition, ethanol consumption was modestly decreased following 3.0 mg/kg naltrexone when compared to 0.3 mg/kg naltrexone [$Z = 1.7$, $p = 0.091$].

There was no difference in the 24 h intake (Fig. 2C).

The 20% ethanol consumption after naltrexone was primarily affected in the MS0 group (Fig. 3). A change in 20% ethanol consumption was observed at 30 min [$\chi^2 = 6.5$, $p = 0.039$] and at 2 h [$\chi^2 = 8.2$, $p = 0.017$] after ethanol access. Compared to saline, both

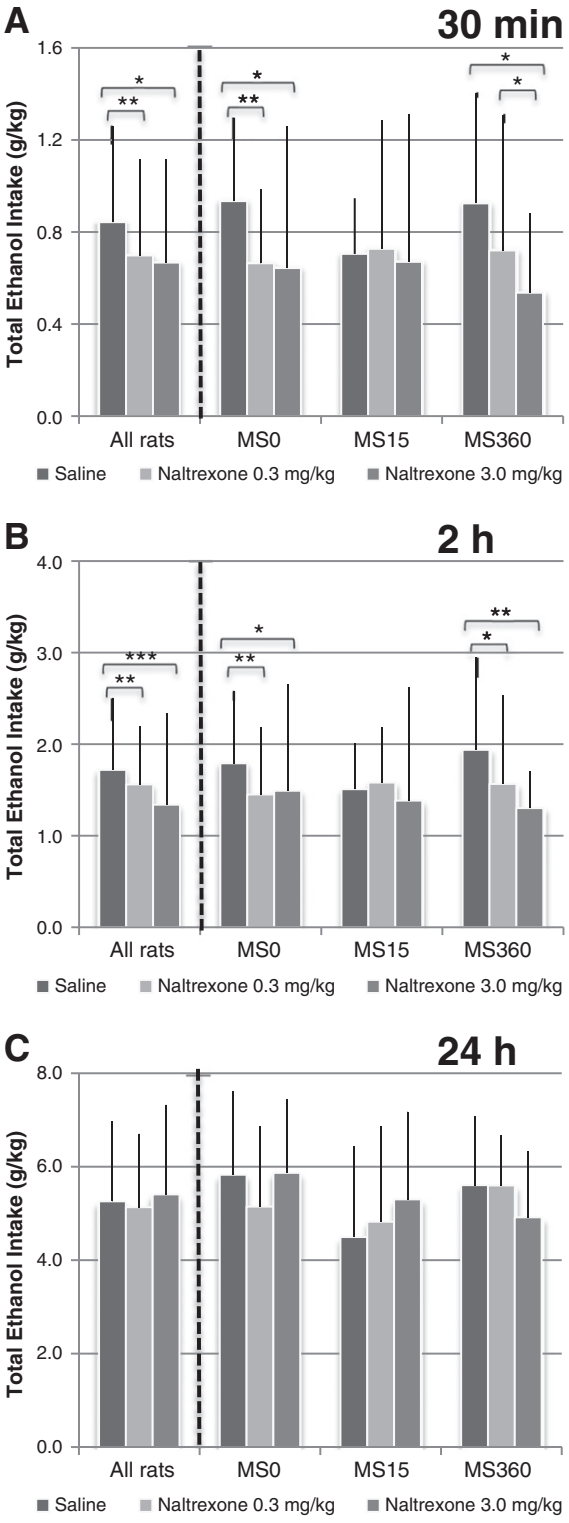

Fig. 1. The total ethanol consumption, *i.e.*, the combined intake of 5% and 20% ethanol (g absolute ethanol/kg body weight), after treatment with saline or naltrexone, 0.3 mg/kg or 3.0 mg/kg, respectively, in rats subjected to three rearing conditions where the pups experienced the absence of the dam for 0, 15 or 360 min (MS0, MS15 or MS360) during postnatal days 1–20. To the left, the naltrexone-induced effects in all rats are shown. To the right, the specific effects in rats exposed to one of three rearing conditions are shown. Ethanol was available 24 h a day, 3 days a week for 6 weeks prior to treatment. During treatment, ethanol intake was measured at 30 min (A), 2 h (B) and 24 h (C) after ethanol access. The injections were made 30 min prior to ethanol access. The data are shown as median ± IQR. MS = maternal separation; $^{*}p \leq 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. (Wilcoxon matched pairs test).

Table 1
Summary of naltrexone-induced effects in rats exposed to different early-life conditions. The table shows the statistical differences in naltrexone-induced effects on voluntary ethanol consumption between groups of rats that were subjected to different early environmental settings; maternal separation for 0 min (MS0), 15 min (MS15) or 360 min (MS360) during postnatal days 1–20. The difference in ethanol intake after naltrexone injections compared to saline injections was calculated for each rat and the naltrexone-induced change was compared between groups with the Kruskal–Wallis test followed by the Mann–Whitney *U*-test.

	Naltrexone (0.3 mg/kg)		Naltrexone (3 mg/kg)	
	30 min intake	2 h intake	30 min intake	2 h intake
	1 h after inj.	2.5 h after inj.	1 h after inj.	2.5 h after inj.
MS0	↓$^{**,\#}$	↓**	↓*	↓*
MS15	=	=	=	=
MS360	=	↓*	↓**	↓*

↓ reduced ethanol consumption compared with saline injections.
= no difference in ethanol consumption compared with saline injections.
* $p<0.05$; ** $p<0.01$ compared to MS15.
$^{\#}$ $p<0.05$ compared to MS360.

doses of naltrexone reduced the consumption of 20% ethanol during the first 30 min (Fig. 3A). Furthermore, there was a reduction in ethanol consumption following 0.3 mg/kg but not 3.0 mg/kg naltrexone (Fig. 3B).

Following naltrexone treatment in the MS15 and MS360 groups, no statistically significant differences in the consumption of 20% ethanol were observed at 30 min and 2 h after ethanol access. Compared to 0.3 mg/kg naltrexone, but not saline, there was a moderate decrease in ethanol consumption 24 h after ethanol access in MS360 rats [$\chi^2 = 6.72$, $p = 0.035$] treated with 3.0 mg/kg naltrexone (Fig. 3C).

The preference for 20% ethanol in relation to the total ethanol consumption (*i.e.* 5 + 20% ethanol) was calculated. If the animals were drinking an equal volume of 5% ethanol and 20% ethanol, then the 20% preference would be 50%. Naltrexone did not change the preference for 20% ethanol in the MS0 or MS360 rats, whereas a trend was observed in the preference for a higher ethanol percentage in the MS15 rats at 30 min [$\chi^2 = 5.6$, $p = 0.060$] and a statistically difference at 2 h [$\chi^2 = 11.5$, $p = 0.003$] (Fig. 4).

3.5. Water intake

Water consumption was not affected in any group after 0.3 mg/kg naltrexone. After administration of 3.0 mg/kg naltrexone, no change was found in the MS15 rats. In the MS0 group, there was a significant decrease in water consumption 2 h after access to ethanol [$\chi^2 = 7.2$, $p = 0.028$], but only after administration of 3.0 mg/kg naltrexone [$Z = 2.4$, $p = 0.015$] when compared to saline. In the MS360 group, a decrease in water intake was observed after 2 h [$\chi^2 = 8.9$, $p = 0.012$]. They had reduced water intake after 3.0 mg/kg [$Z = 2.6$, $p = 0.010$] when compared to 0.3 mg/kg naltrexone.

4. Discussion

The primary, novel finding in the present study is that early environmental experiences determine the ability of naltrexone to reduce ethanol consumption in adult rats. These results provide new insight in the controversy concerning the efficacy of naltrexone in clinical practise. Several studies have described genetic influence on the efficacy of naltrexone (Mague and Blendy, 2010; Monterosso et al., 2001; Oslin et al., 2003) and highlighted the importance of identifying predictors for a robust response to naltrexone (Ray et al., 2010; Rubio et al., 2005; Tidey et al., 2008). In this study, we show the importance of considering not only genetic factors but also early environmental factors when identifying subtypes of AUD patients that respond well to naltrexone treatment.

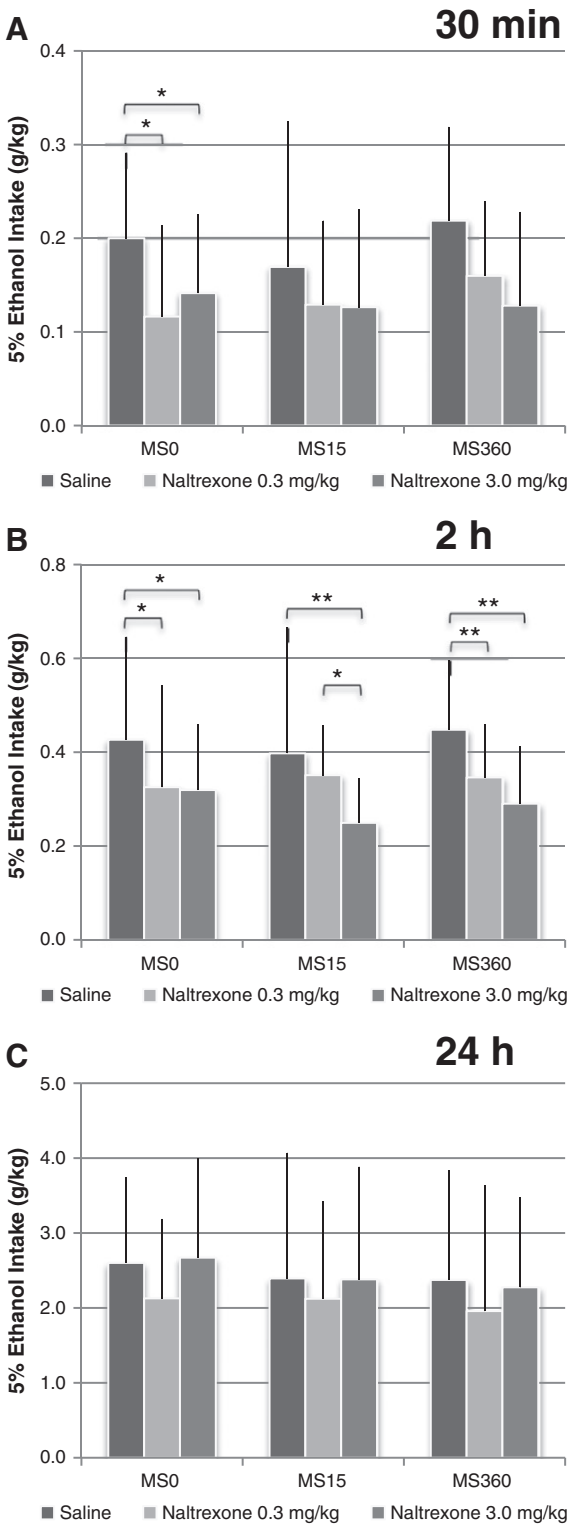

Fig. 2. The consumption of 5% ethanol (g absolute ethanol/kg body weight) after treatment with saline or naltrexone, 0.3 mg/kg or 3.0 mg/kg, respectively, in rats subjected to three rearing conditions where the pups experienced the absence of the dam for 0, 15 or 360 min (MS0, MS15 or MS360) during postnatal days 1–20. Ethanol was available for 24 h a day, 3 days a week for 6 weeks prior to treatment. During treatment, ethanol intake was measured at 30 min (A), 2 h (B) and 24 h (C) after ethanol access. The injections were made 30 min prior to ethanol access. The data are shown as median ± IQR. $^{*}p<0.05$ and $^{**}p\leq0.01$. (Wilcoxon matched pairs test).

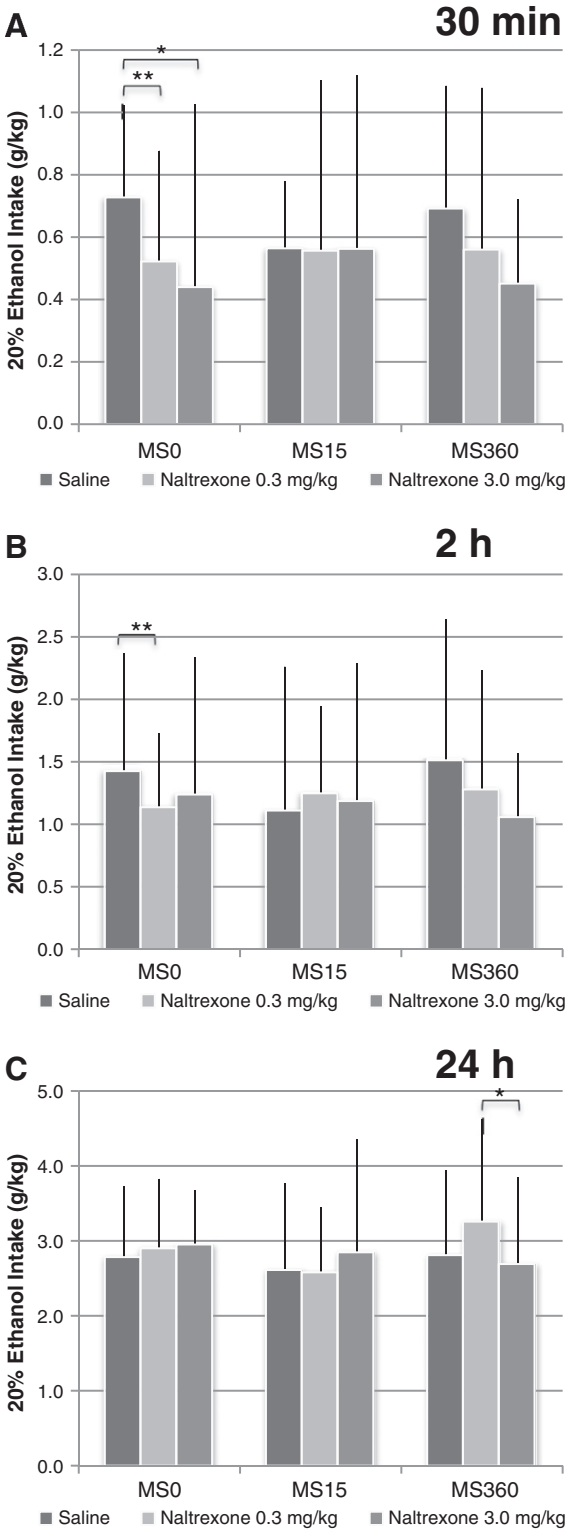

Fig. 3. The consumption of 20% ethanol (g absolute ethanol/kg body weight) after treatment with saline or naltrexone, 0.3 mg/kg or 3.0 mg/kg, respectively, in rats subjected to three rearing conditions where the pups experienced the absence of the dam for 0, 15 or 360 min (MS0, MS15 or MS360) during postnatal days 1–20. Ethanol was available for 24 h a day, 3 days a week for 6 weeks prior to treatment. During treatment, ethanol intake was measured at 30 min (A), 2 h (B) and 24 h (C) after ethanol access. The injections were made 30 min prior to ethanol access. The data are shown as median ± IQR. $^{*}p<0.05$ and $^{**}p\leq0.01$. (Wilcoxon matched pairs test).

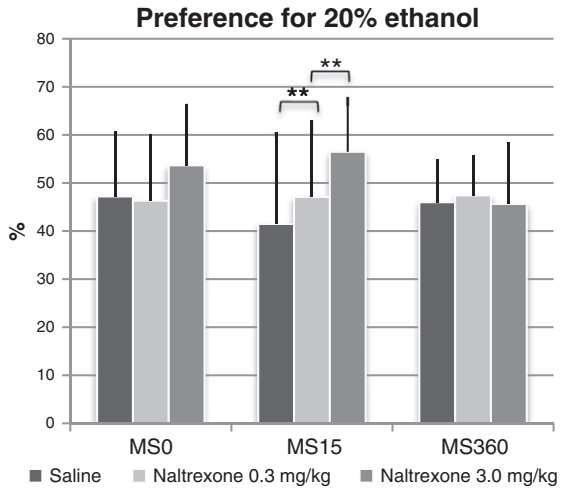

Fig. 4. The preference for 20% ethanol (%) in relation to the total ethanol consumption (5% + 20% ethanol) after 2 h drinking in rats injected with saline or naltrexone, 0.3 mg/kg or 3.0 mg/kg, respectively. The rats were subjected to three rearing conditions where the pups experienced the absence of the dam for 0, 15 or 360 min (MS0, MS15 or MS360) during postnatal days 1–20. Ethanol was available for 24 h a day, three days a week for six weeks prior to treatment. The injections were made 30 min prior to ethanol access. The data are shown as median ± IQR. $^{**}p<0.01$. (Wilcoxon matched pairs test).

Three rearing conditions were used to simulate different early environments. The rat pups that were kept together in litters were deprived of maternal contact for different lengths of time, once daily, during the first postnatal weeks. A number of previous studies have shown that repeated prolonged periods of MS resulted in long-term endocrinological and behavioural consequences, which led to the suggestion that prolonged MS is an adverse rearing condition associated with inability to cope with challenges later in life (Holmes et al., 2005; Ladd et al., 2000; Pryce et al., 2002). The repeated deprivation of maternal contact in the rodent MS model has been used to study the basis for early environmental influence on the vulnerability for psychiatric disorders such as depression and addiction in humans (De Bellis, 2002; Langeland et al., 2004). From studies of ethanol consumption behaviour it is, for example, known that rats experiencing repeated MS for 180–360 min have a propensity for high ethanol intake in adulthood (Gustafsson and Nylander, 2006; Moffett et al., 2007; Roman and Nylander, 2005). In the present study, the MS360 rats were used to simulate early adversity. The control groups differ across MS-studies (Jaworski et al., 2005; Lehmann and Feldon, 2000; Pryce and Feldon, 2003). Commonly used controls include MS0, briefly handled rats, animal facility reared rats or non-handled (NH) rats and the MS outcome is known to be dependent on the control used (Jaworski et al., 2005; Moffett et al., 2007; Roman and Nylander, 2005). In this study, the MS0 rats were briefly handled by the experimenter and used to control for handling effects. Maternal absences for shorter periods of time, such as in the MS15 rats, are considered to mimic wildlife conditions where the dam regularly leaves her litter to forage for food and clearly differ from the continuous presence of the dam that is common in animal facilities (Calhoun, 1963; Fleming and Rosenblatt, 1974). Rat pups kept together in litters but subjected to short repeated MS have been shown to have low adult voluntary ethanol consumption compared to rats that experienced prolonged maternal absence (Gustafsson and Nylander, 2006; Moffett et al., 2007; Roman and Nylander, 2005). In the present study, we used MS15 as a naturalistic rearing paradigm to compare with rearing in a risk environment, MS360.

Recently, a comparative study of MS-induced effects on ethanol intake in adolescent and adult rats showed that the early environment had less impact on drinking behaviour when rats were exposed to free access to ethanol in adolescence instead of adulthood (Daoura et al., 2011). In the present study, the ethanol consumption in a 24-h session was 5–6 g ethanol/kg in all rats, which was a sufficient intake to observe pharmacological effects and naltrexone-induced suppression (Davidson and Amit, 1997; Simms et al., 2008; Wise, 1973). The naltrexone-induced effects could be assessed in individuals with different early environmental histories without confounding effects of large differences in ethanol intake.

Significant differences were observed in the efficacy of naltrexone to reduce the ethanol intake in rats subjected to MS15 and MS360. The total ethanol consumption in the MS15 rats, which were exposed to the most natural and proposed beneficial early environment, was essentially unaffected by naltrexone. These findings suggest that individuals with a history of favourable rearing conditions will not benefit from treatment with naltrexone. However, in the MS15 animals, the intake of 5% ethanol decreased, whereas the preference for 20% ethanol increased with the higher dose of naltrexone. Interestingly, these results were consistent with the increased ethanol intake after naltrexone that was described in individuals with no family history of AUD (Krishnan-Sarin et al., 2007). In contrast, in the MS360 rats, naltrexone treatment reduced the ethanol intake dose-dependently. Previous studies have shown that rats subjected to the MS360 condition have altered risk-assessment and risk-taking behaviour and a blunted stress-induced corticosterone response in adult rats (Roman et al., 2006). Furthermore, adult MS360 rats drink more ethanol (Daoura et al., 2011; Gustafsson and Nylander, 2006; Roman and Nylander, 2005) and prefer higher ethanol concentrations (Gustafsson and Nylander, 2006). These findings suggest that rearing according to MS360 generates an environmentally induced addiction-prone phenotype. The high efficacy of naltrexone in animals reared in a risk environment is of interest when compared to human studies. Clinical reports have described better responses to naltrexone in certain subgroups of patients, such as risk-taking males, individuals with a family history of AUD, early onset AUD, novelty-seeking and impaired impulse control (Gianoulakis, 2004; Kiefer et al., 2008; King et al., 1997; Krishnan-Sarin et al., 2007). Thus, the present results show that the same differences that have been described in individuals with or without hereditary risk factors could be demonstrated in animals exposed either to early risk or to beneficial environmental conditions.

There may be several reasons why MS360 rats, but not MS15 rats, responded with reduced ethanol intake. The different naltrexone-induced effects could, besides the behavioural differences between the groups that were discussed above, be caused by differences in basal neuronal function or ethanol-induced effects on the brain. For example, MS360 may cause the derangement of endogenous opioid networks and may have a different sensitivity to opioid receptor ligands. Long-term MS-induced effects on opioids in MS360 rats (Gustafsson et al., 2008; Ploj et al., 2003) and the different responses to morphine after MS support this theory (Kalinichev et al., 2001a,b). In addition, enhanced naltrexone-induced suppression of sucrose consumption was recently described in rats subjected to prolonged MS (Michaels and Holtzman, 2007). Furthermore, differences in the density of delta and kappa opioid peptide receptors, DOPR and KOPR, respectively, were described in ethanol-drinking MS15 and MS360 rats (Ploj et al., 2003). Because higher doses of naltrexone also affect the DOPR and/or KOPR (Mhatre and Holloway, 2003; Williams and Broadbridge, 2009), differences in these receptors may affect the outcome of naltrexone treatment. Another plausible explanation is the recent finding of different ethanol-induced effects in MS15 and MS360 rats. Rats subjected to MS360 had higher enkephalin levels after voluntary ethanol consumption, whereas no ethanol-induced increase in enkephalins was found in the MS15 rats (Gustafsson et al., 2007). Based on these results, naltrexone abolished the effect of ethanol-induced opioid activation, thereby attenuating the ethanol-induced reward and reducing ethanol intake in the MS360 rats. Similarly, the lack of ethanol-induced activation of opioids in MS15 rats may explain the poor efficacy of

naltrexone in these rats. Further neurobiological studies are necessary to determine the impact of MS and subsequent ethanol exposure on the differences in the drug responses. There is a link between opioid activity and ethanol although it is not clear-cut; both over-activity and opioid deficiency have been associated with vulnerability for AUD (Gianoulakis, 2004; Modesto-Lowe and Fritz, 2005; Oswald and Wand, 2004; Trigo et al., 2010). However, the present and previous results show that environmentally induced changes in endogenous opioids early in life can contribute to individual differences in naltrexone treatment in an animal model. Whether these findings can be translated to humans are at present not known but they are of interest for the understanding of the individual variability in treatment outcome that is seen in the clinic.

The MS0 rats were handled similarly to the other rats but were not separated from the dam. In these rats, we found another pattern of naltrexone responses. Although the rearing conditions were more similar in the MS0 and MS15 rats, the MS0 rats responded similarly to the MS360 rats, which was consistent with previous studies where similar effects were found when comparing MS360 and AFR rats (Gustafsson and Nylander, 2006; Oreland et al., 2009; Roman and Nylander, 2005) and MS360 and MS0 rats (Daoura et al., 2011). However, compared to the MS360 rats, the MS0 rats were more sensitive to naltrexone. They responded to the lower dose with a reduction in the total consumption of 5% and 20% ethanol intake, and they were the only group that decreased their 20% ethanol intake after naltrexone.

It has frequently been reported that naltrexone also affects water consumption, which indicates that naltrexone affects general fluid consumption (Hill et al., 2010; Juarez and Barrios De Tomasi, 2008; Simms et al., 2008). In the present study, water intake was less sensitive to naltrexone than ethanol intake. Only a high dose, 3.0 mg/kg in the MS0 and MS360 rats, affected water intake. Furthermore, in the MS360 rats, water consumption was lower compared to the low dose treatment of naltrexone but not of saline. Thus, the reductions in ethanol intake observed in the present study cannot be solely explained by a general effect on fluid consumption.

In conclusion, ethanol-drinking rats with a history of adverse early environmental experiences responded well to naltrexone, whereas rats reared in an environment related to positive behavioural consequences did not benefit from naltrexone treatment. These results highlight the importance of taking early environmental effects into account when assessing individual differences in treatment outcome and identifying individuals that respond well to naltrexone.

Authors' contribution

Loudin Daoura performed all experiments and data analysis and has actively taken part in planning and writing the manuscript. Ingrid Nylander planned the experiments, took an active part in the analysis of the data, and was the principal investigator in the grants supporting the study.

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