Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 80 (2005) 221–228

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

# Behavioral sensitization and voluntary ethanol drinking in alcohol-preferring AA rats exposed to different regimens of morphine treatment

Sami P. Ojanen, Petri Hyytiä, Kalervo Kiianmaa*

*Department of Mental Health and Alcohol Research, National Public Health Institute, POB 33, 00251 Helsinki, Finland*

Received 1 July 2004; received in revised form 19 October 2004; accepted 13 November 2004
Available online 12 January 2005

**Abstract**

The purpose of the study was to investigate the effects of three different regimens of morphine treatment on subsequent voluntary ethanol drinking in alcohol-preferring AA (Alko Alcohol) rats. The rats were given morphine subcutaneously either intermittently on alternating days (15×10 mg/kg or 5×5–20 mg/kg in escalating doses) or subchronically on four consecutive days (3–20 mg/kg/d). Horizontal locomotor activity was monitored after challenges with additional morphine injections (3 mg/kg) ten days and six weeks after termination of the pretreatment to test if behavioral sensitization was induced by repeated morphine administration. Both intermittent pretreatments induced sensitized locomotor response after the first challenge, whereas subchronic injections did not. After the challenge the rats were given a free choice between tap water and 10% (v/v) ethanol solution for four weeks. The rats pretreated and challenged with morphine did not differ significantly in the acquisition of ethanol drinking from the saline-treated controls. In contrast, ethanol drinking was impaired during the first week of ethanol access in the saline-treated rats given a single morphine injection. The second morphine challenge given after the ethanol-drinking phase did not reveal sensitization in any of the groups. The results suggest that pattern of morphine administration rather than the dose or number of exposures to the drug is the most important factor in induction of behavioral sensitization, and that exposure to ethanol may interfere with this process. They also support earlier findings showing that acute morphine may suppress voluntary ethanol drinking, but failed to provide clear evidence for behavioral sensitization to morphine contributing to predilection towards ethanol in AA rats.


*Keywords:* Alcohol; Alcohol preference; Morphine; Opioids; Self-administration; Selected rat lines; Sensitization

## 1. Introduction

Repeated administration of ethanol and other drugs of abuse results in progressive enhancement of both behavioral and neurochemical responses to the drug. This phenomenon, sensitization, is seen as an expression of long-lasting adaptations in the central nervous system as a result of repeated exposure to a drug (cf. Robinson and Becker, 1986; Stewart and Badiani, 1993). Consequently, evidence has been presented showing that self-administration of drugs of abuse is enhanced in sensitized animals, suggesting that sensitization may be an important process in the development of drug addiction and relapse (Lessov et al., 2001; Lu et al., 2002; Vezina et al., 2002).

Rodent lines differing in ethanol-related phenotypes, such as the alcohol-preferring AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Non-Alcohol) rats selected by bidirectional breeding for high and low voluntary consumption of ethanol, respectively (Eriksson, 1968), have been widely used as tools for identifying behavioral and neuronal mechanisms underlying addiction to ethanol. Recent work has demonstrated that alcohol-preferring AA rats are more susceptible to morphine-induced behavioral and neurochemical sensitization than alcohol-avoiding ANA rats (Honkanen et al., 1999; Mikkola et al., 2002;

* Corresponding author. Tel.: +358 9 4744 8111; fax: +358 9 4744 8133.
*E-mail address:* kalervo.kiianmaa@ktl.fi (K. Kiianmaa).

0091-3057/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.11.012

Ojanen et al., 2003). This finding suggests that there may be a linkage between ethanol consumption and liability to develop sensitization to morphine and possibly to other drugs of abuse, and raises the question whether sensitization is an important mechanism in predilection towards ethanol drinking in AA rats. Furthermore, it is also in line with the view that opioidergic mechanisms contribute to reinforcement from ethanol, and high ethanol preference in AA rats (Hubbell et al., 1986; Hyytiä and Kiianmaa, 2001; Hyytiä and Sinclair, 1993; Stromberg et al., 1997).

The relationship between ethanol consumption and liability to develop sensitization as well as the question of the effect of sensitization on ethanol self-administration seems to be rather complex. The finding that locomotor sensitization was seen in high-alcohol preferring HAP mice but not in low-alcohol preferring LAP mice after repeated injections with ethanol suggests that there is relation between sensitization and ethanol-preference (Grahame et al., 2000). Such a link was not seen in ethanol-preferring C57BL/6 and ethanol-avoiding DBA/2J inbred mice: fewer ethanol exposures were required in DBA/2Js than in C57BL/6s to express locomotor sensitization (Lessov et al., 2001). Interestingly, ethanol drinking, however, was increased in C57BL/6 mice but not in DBA/2J mice after repeated injections of ethanol suggesting that sensitization to the locomotor stimulant effects of ethanol may be associated with increased ethanol intake in mice with high ethanol preference.

One may speculate that morphine-induced sensitization is also expressed in ethanol-related behaviors such as ethanol self-administration. Cross-sensitization between the locomotor stimulant effects of ethanol and those of morphine has been described (Lessov and Phillips, 2003; Nestby et al., 1997). There are also both behavioral and neurochemical data suggesting that opioidergic mechanisms contribute to reinforcement from ethanol and ethanol self-administration. Self-administration studies have shown that, ethanol-preferring AA rats and C57BL/6 mice consume more aqueous solutions of μ-opioid receptor agonists, etonitazene or morphine, than alcohol-avoiding lines or unselected strains (Belknap et al., 1993; Eriksson and Kiianmaa, 1971; Hyytiä and Sinclair, 1993). Moreover, acute administration of morphine has been shown to suppress or increase ethanol drinking in rats depending on the dose, while opioid antagonists can effectively suppress it (Hubbell et al., 1986; Hyytiä and Kiianmaa, 2001; Reid and Hunter, 1984; Sinclair et al., 1973, 1982). In line with the behavioral data, neurochemical studies on opioidergic systems have shown that hypothalamic release of β-endorphin following exposure to ethanol is markedly higher in C57BL/6 mice than in DBA/2 mice (De Waele et al., 1992). Consistently, ethanol elevated the amount of enkephalin precursor peptide more within the nucleus accumbens of AA than ANA rats (Nylander et al., 1994). Furthermore, the distribution of opioid receptors, receptor density, opioid propeptide mRNA levels, as well as G-protein coupled receptor function in various nuclei of the limbic system differs between AA and ANA rats (de Waele et al., 1995; Gianoulakis et al., 1992; Marinelli et al., 2000; Soini et al., 2002). Preclinical findings are supported by data collected from human alcoholics and subjects from families with a history of alcoholism (cf. Gianoulakis, 1996; Sinclair, 2001).

Since behavioral sensitization may be associated with increased drug self-administration in selected rodent lines, and opioidergic systems seem to contribute to reinforcement from ethanol in AA rats, we hypothesized that increased susceptibility to behavioral and neurochemical sensitization may also be important in their high ethanol consumption. Therefore, AA rats exposed to different regimens of morphine treatment were tested for morphine-induced behavioral sensitization and were then given a free choice between tap water and alcohol solution for four weeks. The study evaluated whether the neuroadaptations induced by repeated morphine administration affect the acquisition of voluntary alcohol drinking in the alcohol-preferring AA rats. Since differences have been found between various morphine treatments in their ability to induce sensitization (Powell and Holtzman, 2001; Vanderschuren et al., 1997), our second goal was to examine different regimens of morphine administration in terms of dosage, temporal pattern, and length of treatment for producing behavioral sensitization in AA rats.

## 2. Materials and methods

### 2.1. Animals

Male alcohol-preferring AA (Alko Alcohol) rats (Alcohol Research Centre, National Public Health Institute, Helsinki, Finland) from generation $F_{84}$ were used in the experiments. This rat line together with its counterpart, the alcohol-avoiding ANA (Alko Non-Alcohol) rats, has been outbred from a common founder population by bidirectional selection for high and low voluntary alcohol consumption, respectively (Eriksson, 1968; Sinclair et al., 1989). The experiments were started with three months old AA rats that were housed in groups of four until the measurement of ethanol drinking. Standard maintenance food (SDS RM1 (E) SQC, Witham, Essex, England) and water was freely available, except during the tests for locomotor activity. Ambient temperature was maintained at 22±1 °C and humidity at 55±10%. The rats were kept on 12/12 h light cycle (lights on at 06.00 hours), and they were habituated to handling before starting the experiments. The experiments were performed in compliance with the European Communities Council Directive 86/609/EEC and were approved by the Institutional Animal Care and Use Committee at the National Public Health Institute.

### 2.2. Drugs

Morphine–hydrochloride (University Pharmacy, Helsinki, Finland) was dissolved in isotonic saline (0.9% NaCl, final morphine concentration 3–20 mg/ml). Ethanol (EtOH) was diluted in tap water for a final solution of 10% (v/v).

### 2.3. Morphine treatments

Morphine–HCl or saline was administered subcutaneously in a volume of 1 ml/kg b.w. according to one of the following regimens (Table 1). Fifteen injections of saline were given to saline+saline (SS) and saline+morphine (SM) groups; injections every other day. Five morphine injections in escalating doses were given to group intermittent 5 (IM5); injections every other day (day 1: 5 mg/kg, days 3 and 5: 10 mg/kg, days 7 and 9: 20 mg/kg). The rats belonging to group intermittent 15 (IM15) were given 15 injections of morphine (all 10 mg/kg); injections every other day. Subchronic group CM received seven morphine injections; two injections per day except on day 1 when only one injection was given (day 1: 3 mg/kg, day 2: 2×3 mg/kg, days 3 and 4: 2×10 mg/kg). The total amount of morphine administered was in group IM5 65, in group IM15 150, and in group CM 49 mg/kg. The rats in groups SM, IM5, IM15, and CM were challenged with saline seven days (vehicle challenge), and with morphine (3 mg/kg) ten days (challenge 1, ch1) and six weeks (challenge 2, ch2) after termination of the repeated injections; the rats in group SS received three challenge injections of saline. The first morphine challenge was given ten days after the treatment to avoid any short-term effects of repeated morphine administration. Since the sedative effects of morphine and development of tolerance to them may confuse interpretation of the results, a morphine dose (3 mg/kg), which is predominantly stimulatory, was used in the challenges (ch1 and ch2). Repeated injections were given in the home cages and challenges in the cages used for measuring locomotor activity. Morphine treatments of different groups were conducted in such a way that all rats were tested for sensitization and started and finished their ethanol drinking within two consecutive days.

### 2.4. Locomotor activity

The rats were familiarized to handling and treatment procedures related to measuring of locomotor activity in two measuring sessions without injections (2 h each) during the seven-day period between the completion of the repeated injections and the vehicle challenge session. Horizontal locomotor activity was measured in transparent plastic cages (18×33×15 $cm^3$) by using computer controlled photocells (Cage Rack Activity System, San Diego Instruments, CA, USA). Ambulatory activity in which the rat blocks two or more light beams (7 evenly spaced horizontal beams 6 cm above the base) in rapid succession was used as a measurement for horizontal locomotion. At the beginning of the experiment the rats were weighed, placed into activity cages, and left undisturbed for 15 min to reduce handling-induced activity. After the challenge injection, horizontal locomotor activity was recorded at 10-min intervals for 4 h. Experiments were conducted in a regular colony room with standard lighting. The same procedures were used in all challenge sessions.

### 2.5. Voluntary ethanol drinking

All AA rats used in the experiments were ethanol naïve. About 5 h after receiving the ch1 injection and about an hour after the test for locomotor activity, the rats were placed into single wire mesh cages (21×38×19 $cm^3$) where food, water and 10% (v/v) ethanol were continuously available. Two 100-ml drinking tubes containing tap water or 10% ethanol solution were placed on the front wall of the

Table 1
The regimens of morphine treatment

| Code | Pretreatment | Vehicle challenge | Challenge 1 | EtOH drinking | Challenge 2 |
|---|---|---|---|---|---|
| | 4 days–5 weeks | 7 days after pretreatment | 10 days after pretreatment | 4 weeks | ~6 weeks after pretreatment |
| Saline+saline (SS) | 15 injections of saline (1 ml/kg) every second day | saline | saline | 10% EtOH or water | saline |
| Saline+morphine (SM) | 15 injections of saline (1 ml/kg) every second day | saline | 3 mg/kg | 10% EtOH or water | 3 mg/kg |
| Intermittent 5 (IM5) | 5 escalating doses of morphine: day 1: 5 mg/kg, days 3 and 5: 10 mg/kg, days 7 and 9: 20 mg/kg | saline | 3 mg/kg | 10% EtOH or water | 3 mg/kg |
| Intermittent 15 (IM15) | 15 equal injections of morphine 10 mg/kg, every second day | saline | 3 mg/kg | 10% EtOH or water | 3 mg/kg |
| Subchronic (CM) | 7 escalating doses of morphine, twice a day: day 1: 3 mg/kg, day 2: 2×3 mg/kg, days 3 and 4: 2×10 mg/kg | saline | 3 mg/kg | 10% EtOH or water | 3 mg/kg |

All injections were subcutaneous.
EtOH=ethanol.

cage and the left–right position of the tubes was changed twice a week to avoid any side preference. Ethanol and water consumption were recorded daily, while measurements for food consumption as well as body weight were taken twice a week for four weeks.

### 2.6. Statistical analysis

Ethanol intake (ml) was converted to grams of 100% ethanol/kg body weight for data analyses. Preference scores were calculated as a percentage of consumed ethanol (ml) of the total fluid consumed. Both locomotor activity and ethanol intake were analyzed with mixed-design, 2-way analysis of variance (ANOVA) with treatment (SS, SM, IM5, IM15, CM) as the between-subjects factor and measuring interval (time) as the within-subjects repeated measure. After significant main effect of treatment, pairwise comparisons between the groups were conducted with 2-factor ANOVAs or between means by using post hoc Student–Newman–Keuls (SNK) procedure. Criterion for significance was set at $p<0.05$.

## 3. Results

### 3.1. Locomotor activity

As shown in Fig. 1, locomotor activity of the rats did not differ between the groups following the vehicle challenge given seven days after discontinuation of the repeated injections with morphine [$F(4,35)=1.96$, $p=0.13$, for treatment]. When challenged with morphine, there were significant differences between the treatment groups after the first but not after the second morphine challenge [ch1: $F(4,35)=10.22$, $p<0.001$; ch2: $F(4,35)=1.48$, $p=0.23$]. Further tests (2-way ANOVA) indicated that after the first challenge, all groups given the morphine injection were activated more than the saline group (SS) [$F(1,34)= 19.56$, $p<0.001$, for ch1 injection morphine vs. saline]. Moreover, subsequent post hoc analysis (SNK) between the treatment groups (SS, SM, IM5, IM15 and CM) showed that the groups that had received intermittent morphine treatment (IM5 and IM15), were significantly more activated than the acute morphine group (SM), while the CM group did not differ from the SM group.

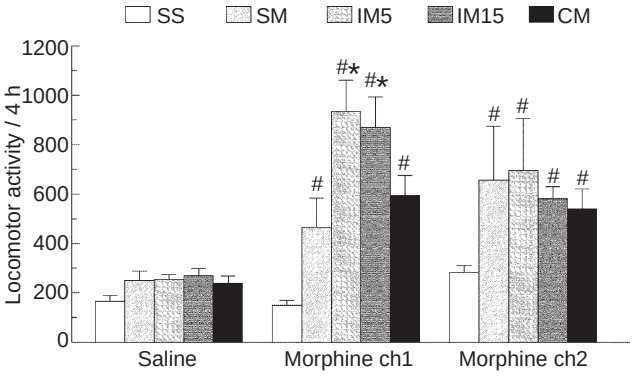


Fig. 1. Locomotor activity in alcohol-preferring AA rats treated repeatedly with saline or morphine according to one of the regimens described in Table 1. Locomotion was measured for 4 h following a challenge injection with saline 7 days or morphine (3 mg/kg) 10 days (ch 1) and 6 weeks (ch 2) after termination of the repeated injections. #$p<0.05$, relative to saline group (SS), *$p<0.05$, relative to SM group, Student–Newman–Keuls $t$-test. Mean photocell counts±S.E.M., $N$=5–8.

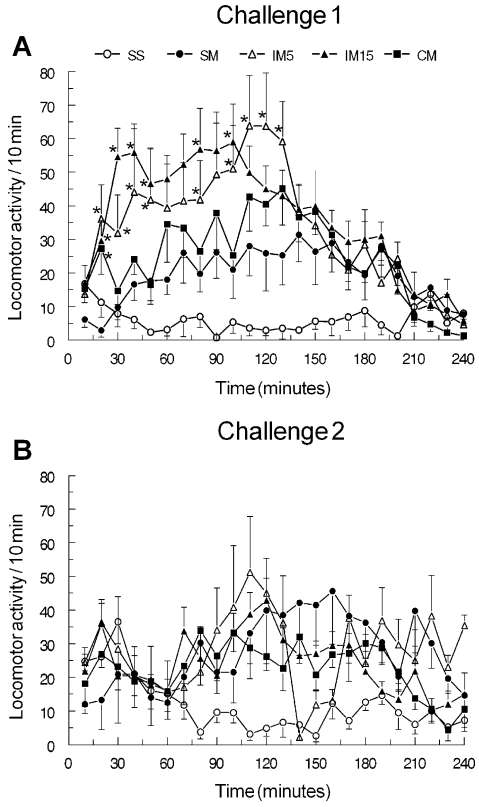


Fig. 2. Effects of morphine (3 mg/kg, s.c.) on locomotor activity in alcohol-preferring AA rats treated repeatedly with saline or morphine according to one of the regimens described in Table 1. The rats were challenged with morphine 10 days (ch1, upper panel) and 6 weeks (ch2, lower panel) after termination of the sensitizing treatment. *$p<0.05$, relative to saline group (SM), Student–Newman–Keuls $t$-test. Mean photocell counts±S.E.M., $N$=5–8.

Fig. 2 shows the pattern of locomotor activity during the morphine challenge sessions. Morphine (ch1, ch2) stimulated locomotor activity in all groups compared with saline challenged controls across the experiment, 15–210 min after the injection [ch1: $F(1,34)=19.56$, $p<0.001$; ch2: $F(1,34)=5.70$, $p=0.023$, for treatment across the 240-min period]. The peak of activity in all morphine challenged groups was recorded approximately 120–150 min after the injection (ch1). Two-way ANOVAs on pairs of groups of the first challenge (ch1) showed that the IM5 and IM15 rats were activated more than the SM [IM5: $F(23,253)=4.13$, $p<0.001$; IM15: $F(23,299)=3.82$, $p<0.001$, for treatment×time] and CM rats [IM5: $F(23,253)=1.76$, $p=0.019$; IM15: $F(23,299)=2.41$, $p<0.001$, for treatment×time](Fig. 2A). The CM group did not differ from the SM group.

When challenged for the second time (ch2), there were no significant differences among the groups injected with morphine. Moreover, the activity of SM controls was compared between the challenges 1 and 2 in order to show possible residual effect of the previous morphine exposure.

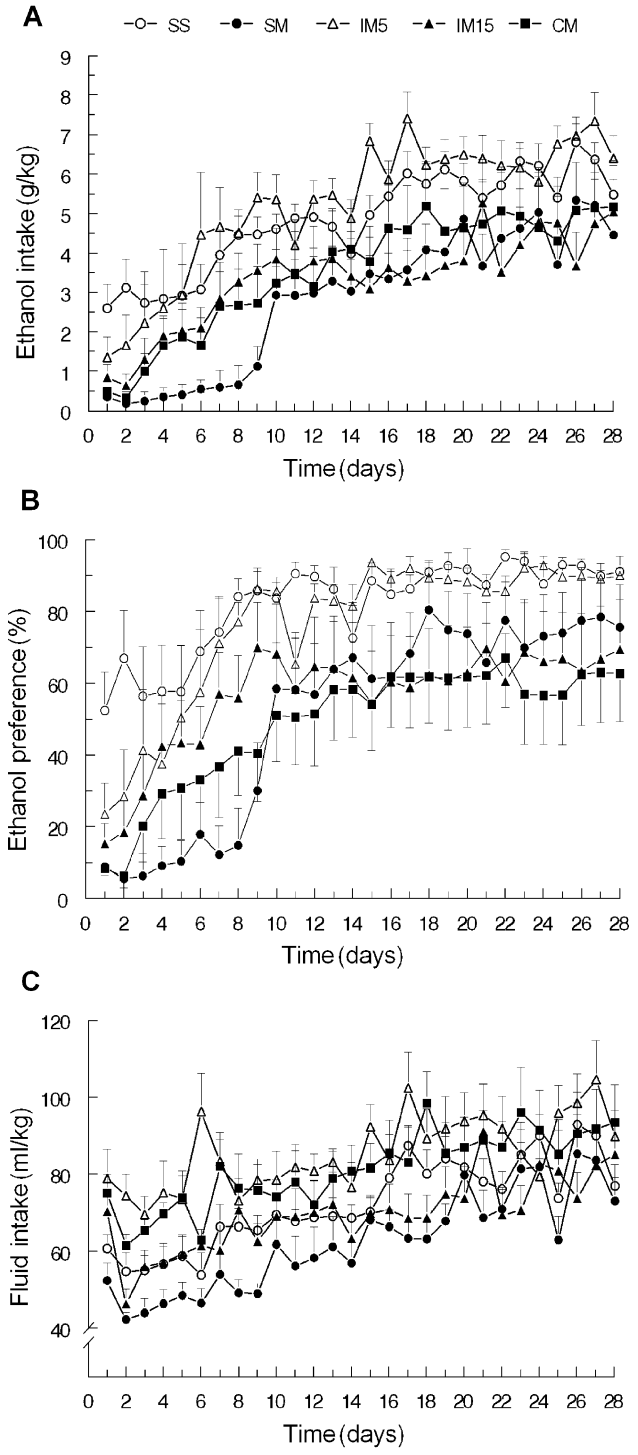


Fig. 3. Daily voluntary ethanol drinking in g/kg b.w. (panel A), ethanol preference (panel B) and total fluid intake (panel C) in alcohol-preferring AA rats treated repeatedly with saline or morphine according to one of the regimens described in Table 1. Means±S.E.M., $N$=5–8.

Significant difference was not found between the challenges [$F(1,7)=1.99$, $p=0.20$, for challenge].

### 3.2. Voluntary ethanol drinking

Daily consumption of ethanol in g/kg across the 28 days of the experiment is shown in Fig. 3A. An ANOVA of the results (28 d) indicated a tendency for differential ethanol drinking among the groups [$F(4,31)=2.50$, $p=0.063$, for treatment; $F(108,837)=1.25$, $p=0.053$, for treatment×time interaction]. Hence, a more detailed comparison of the weekly drinking levels revealed a significant main effect for treatment [$F(4,31)=3.79$, $p=0.013$] during the first week of ethanol access, but not for the second [$F(4,31)=2.18$, $p=0.095$], third [$F(4,31)=2.12$, $p=0.102$] or fourth week [$F(4,31)=1.02$, $p=0.412$]. Therefore, pairwise comparisons using 2-way ANOVAs with time as the within-subjects repeated measure were conducted only for the first week data. These analyses showed that ethanol intake by the SM group only differed significantly from the SS rats [$F(1,14)=14.65$, $p=0.02$, for treatment]. Moreover, ethanol intake by the SM rats was also lower than that by the IM5, IM15 and CM rats [IM5: $F(1,12)=6.67$, $p=0.024$; IM15: $F(1,14)=12.378$, $p=0.003$; CM: $F(1,15)=8.35$, $p=0.011$, for treatment].

Fig. 3B shows the average daily ethanol preference ratios. Ethanol preference reached almost 90% in the SS and IM5 groups within two weeks and was maintained at this level thereafter. In contrast, in the SM, IM15, and CM groups ethanol preference was maintained at 60–80% after reaching this level. Comparisons of the preference ratios across the 28 days showed significant main effects for both treatment [$F(4,31)=2.84$, $p=0.041$] and treatment×time interaction [$F(108,837)=1.72$, $p<0.001$]. Further analysis of the data between individual groups with 2-factor ANOVAs with repeated measures on time revealed that ethanol preference was significantly lower in the SM and CM rats [SM: $F(1,14)=11.20$, $p=0.005$; CM: $F(1,14)=7.46$, $p=0.016$, for treatment] than in the SS rats, showing that the lower intake of ethanol in SM rats was not only due to reduced intake of all fluids (Fig. 3C). In fact, total fluid intake in SM rats increased across the experiment. Furthermore, ethanol preference in the IM5 group was significantly higher than in the SM group [$F(1,11)=5.28$, $p=0.042$, for treatment].

## 4. Discussion

Repeated administration of various drugs of abuse, including ethanol and morphine, leads to an increase of their stimulatory effects; a phenomenon called behavioral sensitization (Babbini and Davis, 1972; Nestby et al., 1997; Phillips et al., 1994). It can also be induced by mild stressors such as isolation or food deprivation (cf. Kalivas and Stewart, 1991; Marinelli and Piazza, 2002). Interestingly,

cross-sensitization between the locomotor stimulant effects of ethanol and those of morphine has been described (Lessov and Phillips, 2003; Nestby et al., 1997). There are also data suggesting that sensitization contributes to the development of drug addiction and promotes drug-seeking behavior (Lu et al., 2002; Vezina et al., 2002).

The present study conducted in alcohol-preferring AA rats showed that intermittent morphine treatments induced sensitization of locomotor activity, but subsequent acquisition of ethanol drinking behavior was not different from that in the saline-treated controls. Subchronic treatment with morphine was unable to either induce behavioral sensitization or affect acquisition of ethanol drinking. Furthermore, none of the groups showed sensitized locomotor response to morphine when challenged again after four weeks of ethanol drinking.

According to our results, only the intermittent drug administration was able to induce sensitization to the locomotor stimulant effects of morphine in AA rats. The data are in line with the results published by other authors suggesting that pattern of exposure rather than the dose administered is important in the development of behavioral sensitization (Powell and Holtzman, 2001; Vanderschuren et al., 1997). Sensitization was evident in animals that were treated intermittently with morphine (groups IM5 and IM15). There was no difference in the amount of behavioral sensitization between the two groups although the total dose of morphine administered, number of injections as well as the length of the treatment was lower in the IM5 than in the IM15 group. Only a tendency for increased activation was seen in the group CM receiving morphine subchronically, although similar treatment has been found to produce behavioral sensitization elsewhere (Powell and Holtzman, 2001). While the total dose of morphine given to the CM rats was in the same range as in the IM5 rats and the number of drug exposures was even higher, it cannot be definitely ruled out that the CM rats were not sensitized because they did not meet a certain threshold dose of morphine or length of treatment needed to induce behavioral sensitization. However, this seems unlikely since previous studies have shown behavioral and neurochemical sensitization as a consequence of a single 10 mg/kg morphine injection in rats (Vanderschuren et al., 2001).

The second challenge with morphine given after four weeks ethanol drinking and about six weeks after discontinuation of the pretreatment did not induce a sensitized response in any of the groups. Our previous studies showed that behavioral sensitization in AA rats is very long-lasting, and consequently we expected the rats to show a sensitized response also in the present study (Ojanen et al., 2003). In contrast to the previous study, the rats were here tested for acquisition of ethanol drinking between the first and second challenge with morphine. These findings thus raise the possibility that ethanol drinking following morphine treatment interfered with the neuronal processes underlying morphine-induced behavioral sensitization. In line with this view, Kosten and Bombace (2000) reported that intraperitoneal injections of ethanol given during repeated treatment with morphine attenuated locomotor sensitization to morphine. Because the same was true in animals receiving morphine and the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801, they concluded that ethanol may alter plasticity effects of repeated morphine administration because of its NMDA antagonist properties. In another study, chronic voluntary ethanol drinking suppressed nicotine-induced behavioral sensitization in rats (Darbra et al., 2004).

It is also possible that environmental conditions, such as stress caused by isolation housing during the ethanol-drinking phase, altered neural responses to morphine and resulted in the attenuation of the sensitized response to the second challenge in the present experiment. Earlier data on the interaction between different stressors and sensitization, however, suggest that stress predominantly increases morphine-induced locomotion (del Rosario et al., 2002; Deroche et al., 1994; Stöhr et al., 1999). Therefore, it seems improbable that stress related to housing or some other environmental conditions can explain the absence of enhanced behavioral response during the second challenge.

The idea that sensitization may contribute to ethanol self-administration has gained support from some recent findings by other authors. Lessov et al. (2001) showed that C57Bl/6 mice sensitized to ethanol consumed more ethanol than their saline-treated controls. On the other hand, this did not seem to be true for ethanol-avoiding animals, since DBA/2J mice consumed little ethanol despite of sensitization of ethanol-induced locomotion. Opioidergic mechanisms that have been implicated in the mediation and modulation of ethanol reinforcement (cf. Gianoulakis, 2001; Herz, 1997), could contribute also to enhanced ethanol preference in sensitized animals. This is suggested by findings showing that behavioral sensitization to ethanol in mice can be prevented by co-administration of opioid antagonist naltrexone (Camarini et al., 2000), and that repeated injections with morphine may increase ethanol drinking or preference (Hodge et al., 1992; Volpicelli et al., 1991). These findings led us to hypothesize that susceptibility to morphine-induced behavioral sensitization in AA rats is related and contributes to the acquisition of and the predilection towards ethanol drinking in AA rats.

In contrast to this hypothesis, our results indicated that ethanol drinking by the rats treated repeatedly with morphine was not different from that by the SS controls whether it was expressed as g/kg/d (IM5, IM15, CM) or as a preference ratio (IM5, IM15). Acquisition of ethanol drinking and ethanol preference were, nonetheless, impaired during the first week in the saline-treated SM rats injected with morphine only once. The long-lasting suppression of drinking by the 3 mg/kg morphine dose is surprising because similar doses have been shown to stimulate

locomotor activity in the AA rats in the present and previous studies (Honkanen et al., 1999) and to increase ethanol intake (Reid and Hunter, 1984). It should be noted, however, that the ethanol drinking session started here 6 h after the acute morphine injection, when the morphine naïve animals were possibly experiencing aversive after-effects of morphine administration. These effects may have been associated with ethanol availability, and therefore the acquisition of ethanol drinking was delayed in this group compared with the groups that had received morphine injections repeatedly and had developed partial tolerance to the aversive effects.

The failure to see enhancement of ethanol drinking in the groups sensitized with morphine seems to contradict with the studies where repeated injections of morphine increased ethanol intake in rats (Hodge et al., 1992; Stromberg et al., 1997; Volpicelli et al., 1991). However, the general picture emerging from these studies is that morphine, whether given acutely or repeatedly, does not affect acquisition of ethanol drinking, but increases ethanol drinking during the maintenance phase. Besides, the comparison of the previous studies with our design is difficult, because we do not know whether repeated morphine administration resulted in sensitization in them. In any case, our results support the notion that during initial exposure to ethanol, stimulation of the endogenous opioid system with repeated morphine injections does not enhance learning of the reward value of ethanol. Further studies are warranted to investigate the role of sensitization in acquired ethanol intake or in intake stimulated by opioids.

## Acknowledgements

We thank Ms. Leena Tanner-Väisänen and Ms. Pirkko Johansson for their technical assistance. This work was supported by a grant from the Finnish Foundation for Alcohol Studies to Sami Ojanen.

## References

Babbini M, Davis WM. Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. Br J Pharmacol 1972;46(2):213–24.

Belknap JK, Crabbe JC, Riggan J, O'Toole LA. Voluntary consumption of morphine in 15 inbred mouse strains. Psychopharmacology (Berl) 1993;112(2–3):352–8.

Camarini R, Nogueira Pires ML, Calil HM. Involvement of the opioid system in the development and expression of sensitization to the locomotor-activating effect of ethanol. Int J Neuropsychopharmacol 2000;3(4):303–9.

Darbra S, Pallares M, Ferre N. Effects of voluntary alcohol intake on nicotine-induced behavioural sensitisation in rats. Pharmacol Biochem Behav 2004;77(4):815–22.

del Rosario CN, Pacchioni AM, Cancela LM. Influence of acute or repeated restraint stress on morphine-induced locomotion: involvement of dopamine, opioid and glutamate receptors. Behav Brain Res 2002;134(1–2):229–38.

Deroche V, Piazza PV, Le Moal M, Simon H. Social isolation-induced enhancement of the psychomotor effects of morphine depends on corticosterone secretion. Brain Res 1994;640(1–2):136–9.

de Waele JP, Papachristou DN, Gianoulakis C. The alcohol-preferring C57BL/6 mice present an enhanced sensitivity of the hypothalamic beta-endorphin system to ethanol than the alcohol-avoiding DBA/2 mice. J Pharmacol Exp Ther 1992;261(2):788–94.

de Waele JP, Kiianmaa K, Gianoulakis C. Distribution of the mu and delta opioid binding sites in the brain of the alcohol-preferring AA and alcohol-avoiding ANA lines of rats. J Pharmacol Exp Ther 1995;275(1):518–27.

Eriksson K. Genetic selection for voluntary alcohol consumption in the albino rat. Science 1968;159:739–41.

Eriksson K, Kiianmaa K. Genetic analysis of susceptibility to morphine addiction in inbred mice. Ann Med Exp Biol Fenn 1971;49(2):73–8.

Gianoulakis C. Implications of endogenous opioids and dopamine in alcoholism: human and basic science studies. Alcohol Alcohol, Suppl 1996;1:33–42.

Gianoulakis C. Influence of the endogenous opioid system on high alcohol consumption and genetic predisposition to alcoholism. J Psychiatry Neurosci 2001;26(4):304–18.

Gianoulakis C, de Waele JP, Kiianmaa K. Differences in the brain and pituitary beta-endorphin system between the alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol, Clin Exp Res 1992;16(3):453–9.

Grahame NJ, Rodd-Henricks K, Li TK, Lumeng L. Ethanol locomotor sensitization, but not tolerance correlates with selection for alcohol preference in high- and low-alcohol preferring mice. Psychopharmacology (Ber) 2000;151(2–3):252–60.

Herz A. Endogenous opioid systems and alcohol addiction. Psychopharmacology (Berl) 1997;129(2):99–111.

Hodge CW, Andrews CM, Lewis RS, Samson HH. Effects of morphine on acquisition and maintenance of ethanol and water intake patterns in rats. Alcohol 1992;9(5):433–40.

Honkanen A, Mikkola J, Korpi ER, Hyytiä P, Seppälä T, Ahtee L. Enhanced morphine- and cocaine-induced behavioral sensitization in alcohol-preferring AA rats. Psychopharmacology (Berl) 1999;142(3):244–52.

Hubbell CL, Czirr SA, Hunter GA, Beaman CM, LeCann NC, Reid LD. Consumption of ethanol solution is potentiated by morphine and attenuated by naloxone persistently across repeated daily administrations. Alcohol 1986;3(1):39–54.

Hyytiä P, Kiianmaa K. Suppression of ethanol responding by centrally administered CTOP and naltrindole in AA and Wistar rats. Alcohol, Clin Exp Res 2001;25(1):25–33.

Hyytiä P, Sinclair JD. Oral etonitazene and cocaine consumption by AA, ANA and Wistar rats. Psychopharmacology (Berl) 1993;111(4):409–14.

Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev 1991;16(3):223–44.

Kosten TA, Bombace JC. Prior and delayed applications of dizocilpine or ethanol alter locomotor sensitization to morphine. Brain Res 2000;878(1–2):20–31.

Lessov CN, Phillips TJ. Cross-sensitization between the locomotor stimulant effects of ethanol and those of morphine and cocaine in mice. Alcohol, Clin Exp Res 2003;27(4):616–27.

Lessov CN, Palmer AA, Quick EA, Phillips TJ. Voluntary ethanol drinking in C57BL/6J and DBA/2J mice before and after sensitization to the locomotor stimulant effects of ethanol. Psychopharmacology (Berl) 2001;155(1):91–9.

Lu L, Xu NJ, Ge X, Yue W, Su WJ, Pei G, et al. Reactivation of morphine conditioned place preference by drug priming: role of environmental cues and sensitization. Psychopharmacology (Berl) 2002;159(2):125–32.

Marinelli M, Piazza PV. Interaction between glucocorticoid hormones, stress and psychostimulant drugs. Eur J Neurosci 2002;16(3):387–94.

Marinelli PW, Kiianmaa K, Gianoulakis C. Opioid propeptide mRNA content and receptor density in the brains of AA and ANA rats. Life Sci 2000;66(20):1915–27.

Mikkola JA, Janhunen S, Hyytiä P, Kiianmaa K, Ahtee L. Rotational behaviour in AA and ANA rats after repeated administration of morphine and cocaine. Pharmacol Biochem Behav 2002;73(3): 697–702.

Nestby P, Vanderschuren LJ, De Vries TJ, Hogenboom F, Wardeh G, Mulder AH, et al. Ethanol, like psychostimulants and morphine, causes long-lasting hyperreactivity of dopamine and acetylcholine neurons of rat nucleus accumbens: possible role in behavioural sensitization. Psychopharmacology (Berl) 1997;133(1):69–76.

Nylander I, Hyytiä P, Forsander O, Terenius L. Differences between alcohol-preferring (AA) and alcohol-avoiding (ANA) rats in the prodynorphin and proenkephalin systems. Alcohol, Clin Exp Res 1994;18(5):1272–9.

Ojanen S, Koistinen M, Bäckström P, Kankaanpää A, Tuomainen P, Hyytiä P, et al. Differential behavioural sensitization to intermittent morphine treatment in alcohol-preferring AA and alcohol-avoiding ANA rats: role of mesolimbic dopamine. Eur J Neurosci 2003;17(8):1655–63.

Phillips TJ, Dickinson S, Burkhart-Kasch S. Behavioral sensitization to drug stimulant effects in C57BL/6J and DBA/2J inbred mice. Behav Neurosci 1994;108(4):789–803.

Powell KR, Holtzman SG. Parametric evaluation of the development of sensitization to the effects of morphine on locomotor activity. Drug Alcohol Depend 2001;62(1):83–90.

Reid LD, Hunter GA. Morphine and naloxone modulate intake of ethanol. Alcohol 1984;1(1):33–7.

Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 1986;396(2): 157–98.

Sinclair JD. Evidence about the use of naltrexone and for different ways of using it in the treatment of alcoholism. Alcohol Alcohol 2001; 36(1):2–10.

Sinclair JD, Adkins J, Walker S. Morphine-induced suppression of voluntary alcohol drinking in rats. Nature 1973;246(5433):425–7.

Sinclair JD, Rusi M, Airaksinen MM, Altshuler HL. Relating TIQ's, opiates, and ethanol. Prog Clin Biol Res 1982;90:365–76.

Sinclair JD, Le AD, Kiianmaa K. The AA and ANA rat lines, selected for differences in voluntary alcohol consumption. Experientia 1989;45(9): 798–805.

Soini SL, Hyytiä P, Korpi ER. Brain regional μ-opioid receptor function in rat lines selected for differences in alcohol preference. Eur J Pharmacol 2002;448(2–3):157–63.

Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 1993;4(4):289–312.

Stöhr T, Almeida OF, Landgraf R, Shippenberg TS, Holsboer F, Spanagel R. Stress- and corticosteroid-induced modulation of the locomotor response to morphine in rats. Behav Brain Res 1999;103(1):85–93.

Stromberg MF, Meister SC, Volpicelli JR, Ulm RR. Low dose of morphine and the consumption of a sweetened ethanol solution: differential effects on acquisition and maintenance. Alcohol 1997;14(5):463–8.

Vanderschuren LJ, Tjon GH, Nestby P, Mulder AH, Schoffelmeer AN, De Vries TJ. Morphine-induced long-term sensitization to the locomotor effects of morphine and amphetamine depends on the temporal pattern of the pretreatment regimen. Psychopharmacology (Berl) 1997;131(2): 115–22.

Vanderschuren LJ, De Vries TJ, Wardeh G, Hogenboom FA, Schoffelmeer AN. A single exposure to morphine induces long-lasting behavioural and neurochemical sensitization in rats. Eur J Neurosci 2001;14(9): 1533–8.

Vezina P, Lorrain DS, Arnold GM, Austin JD, Suto N. Sensitization of midbrain dopamine neuron reactivity promotes the pursuit of amphetamine. J Neurosci 2002;22(11):4654–62.

Volpicelli JR, Ulm RR, Hopson N. Alcohol drinking in rats during and following morphine injections. Alcohol 1991;8(4):289–92.