Morphine Decreases Social Interaction of Adult Male Rats, While THC Does Not Affect It

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Summary

The aim of the present study was to compare effect of three low doses of morphine (MOR) and delta9-tetrahydrocannabinol (THC) on social behavior tested in Social interaction test (SIT). 45 min prior to testing adult male rats received one of the drugs or solvents: MOR (1; 2.5; 5 mg/kg); saline as a solvent for MOR; THC (0.5; 1; 2 mg/kg); ethanol as a solvent for THC. Occurrence and time spent in specific patterns of social interactions (SI) and non-social activities (locomotion and rearing) was video-recorded for 5 min and then analyzed. MOR in doses of 1 and 2.5 mg/kg displayed decreased SI in total. Detailed analysis of specific patterns of SI revealed decrease in mutual sniffing and allo-grooming after all doses of MOR. The highest dose (5 mg/kg) of MOR decreased following and increased genital investigation. Rearing activity was increased by lower doses of MOR (1 and 2.5 mg/kg). THC, in each of the tested doses, did not induce any specific changes when compared to matching control group (ethanol). However, an additional statistical analysis showed differences between all THC groups and their ethanol control group when compared to saline controls. There was lower SI in total, lower mutual sniffing and allo-grooming, but higher rearing in THC and ethanol groups than in saline control group. Thus, changes seen in THC and ethanol groups are seemed to be attributed mainly to the effect of the ethanol. Based on the present results we can assume that opioids affect SI more than cannabinoid.

Key words

Social behavior • Opioids • Morphine • Cannabinoids • THC • Male rats

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Introduction

Psychotropic drugs are known to have serious influence on humans' as well as animals' behavior (Hayase *et al.* 2005, Šlamberová 2012, Šlamberová *et al.* 2014). In humans drugs evoke positive emotions of joy and happiness, or suppress negative states of anxiety or type of depression (Nesse and Berridge 1997, Šlamberová *et al.* 2015b). Even though drugs of abuse are usually interpreted as socializing substances, because they are often used in dancing clubs and parties, the opposite may be true.

Many studies (Ando *et al.* 2006, Miczek and Tidey 1989, Trezza *et al.* 2014), including our own (Šlamberová *et al.* 2010b, Šlamberová *et al.* 2015a), repeatedly demonstrate that psychostimulant drugs in rats decrease social behavior. For example acute treatment with high doses of MDMA (3,4-methylenedioxymethamphetamine) inhibits social play behavior (Homberg *et al.* 2007) and decreases social investigation (Daza-Losada *et al.* 2009, Maldonado and Navarro 2001, Navarro and Maldonado 1999). Our previous studies have demonstrated that psychostimulants, such as methamphetamine, amphetamine, cocaine and MDMA, suppress social

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interaction (SI) in adult male rats in a dose-specific manner (Šlamberová *et al.* 2010b, Šlamberová *et al.* 2015a). Further, we demonstrated that the environmental conditions such as familiarity of the experimental arena and intensity of light play a role in both social and non-social activities (Šlamberová *et al.* 2010b). While the SI was decreased, locomotion and exploratory rearing were increased in the unfamiliar arena suggesting higher interest in the exploration of the unknown environment without any interest in the conspecific (Šlamberová *et al.* 2010b).

To extend our previous findings, the present study was targeted on effects of psychostimulants with different mechanism of action on SI in order to extend our previous findings. Therefore, opioids as the "hard" drugs with a rich history and cannabinoids as the "light" drug popular in young people were investigated in the present study.

Animal studies from the '80s and '90s of the past century showed that opioids are very effective in reducing social separation-induced distress vocalizations in pups, they decrease proximity maintenance time in socially housed animals, increase play and decrease maternal aggression (Panksepp *et al.* 1980). Exposure to morphine (MOR) *in utero* was shown to increase social play in adolescent rodents (Niesink *et al.* 1996) and increase appetite, while decreasing effectiveness of sexual behavior (Vathy 1993). On the other hand, our previous study (Šlamberová *et al.* 2001) demonstrated that MOR administration during the second half of pregnancy attenuates some components of maternal behavior, while increasing other "non-maternal" activities of mothers.

Similarly, cannabinoids have also various effects on social behavior depending on type of cannabinoid drug, dose and test used for evaluation of the effects (Katsidoni *et al.* 2013). Delta9-tetrahydrocannabinol (THC) affecting cannabinoid CB1 receptors, but not cannabidiol that is inverse agonist of cannabinoid CB2 receptors, reduces contact and aggressive behavior (Klein *et al.* 2011, van Ree *et al.* 1984). In addition, O'Shea (2006) demonstrated decreased SI after chronic treatment with the cannabinoid receptor agonist (CP 55 940) following a 28-day drug-free period before the test. Further, Schneider (2008) demonstrated that acute cannabinoid administration induced more deficits in social behavior of pubertal rats than in mature rats.

The aim of the present study was to compare the effect of three low doses of MOR, as a representative of opioids, and three low doses of THC, as a representative of cannabinoids, on social behavior tested in the Social interaction test (SIT).

Methods

Animals

In total 65 adult male albino Wistar rats (8-9 pair per group) were delivered by Velaz (Prague, the Czech Republic) from Charles River Laboratories International, Inc. Animals were housed four per cage and left undisturbed for a week in a temperature-controlled (22-24 ºC) colony room with free access to food and water on a 12 h (light) : 12 h (dark) cycle with lights on at 06:00 h.

Social interaction test (SIT)

Animals (always one animal per time) were first habituated individually in the Open field (45x45x30 cm) on two consecutive days in a dimly lit room for 10 min to get familiar with the arena (File and Hyde 1978). On the third day, a pair of unfamiliar animals (each from different home-cage) of the same weight and treatment was tested for SI. The experiment took place in the same condition as habituation. The injection of drug and their vehicles (see below) was applied subcutaneously (s.c.) 45 min prior to the SIT.

The behavior of each pair of animals was videorecorded for 5 min. Subsequently, recordings were evaluated offline by means of ODLog software (Macropod Software). The number (occurrence) and the time spent in SI and non-social behaviors were evaluated. Analyzed parameters were as follows: 1) SI included the following patterns: sniffing (mutual), genital investigation (sniffing the anogenital area), following (the pursuit of one animal by another), climbing over and crawling under the partner and allo-grooming; 2) non-social behavior: locomotion (horizontal exploration) and rearing (vertical exploration).

Drug treatment

Drugs, MOR (1.0; 2.5; 5.0 mg/kg) or THC (0.5; 1.0; 2.0 mg/kg), respectively, were administered s.c. 45 min prior to SIT. MOR was dissolved in distilled water and THC in ethanol. Control group for MOR received injection of 0.9 % saline and control group for THC 7.2 % ethanol. The administered volume was always 1 ml/kg. These doses were chosen based on our preliminary data showing that these doses do not induce

stereotypy behavior. The interval of 45 min was chosen based on our published (Rambousek *et al.* 2014) and some unpublished pharmacokinetic data showing that the peak of the drugs in the brain (not in the blood) is between 45th and 60th minute after s.c. administration.

Statistical analysis

Effects of each drug (MOR and THC) were analyzed separately. SI in total was analyzed first, and then the particular patterns of social and non-social behaviors. A One-way ANOVA (drug treatment) with the Bonferroni *post-hoc* test was used to analyze the time spent in and number of the particular types of social and non-social behaviors. Because no differences were found between THC and their ethanol control, an additional statistical analysis was run to see differences between THC, ethanol and saline control groups. Differences were considered significant if $p<0.05$.

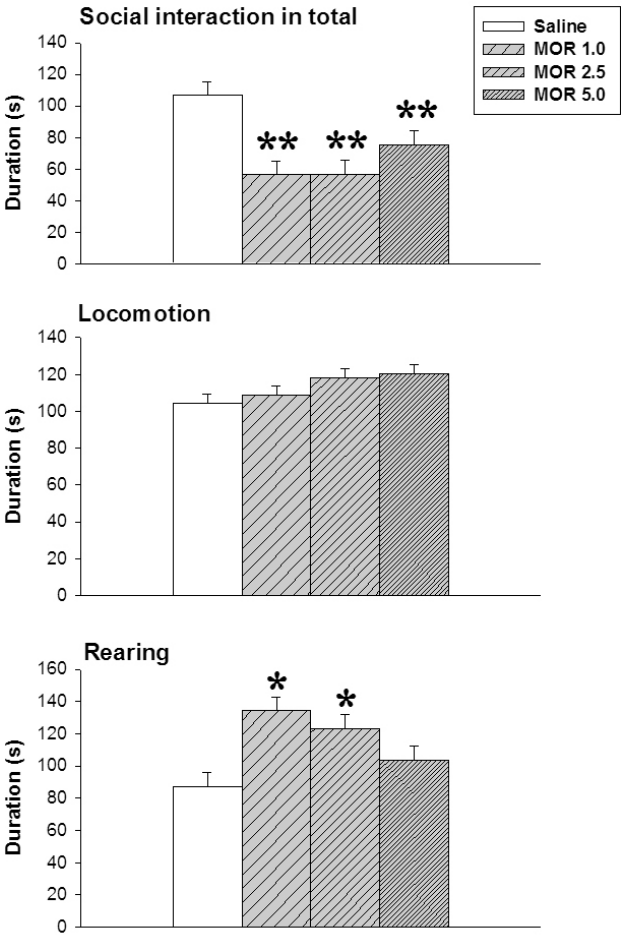

Fig. 1. The effect of morphine on social interaction in total and locomotor and exploratory behavior of adult male rats. Values are mean ± SEM (n=8-9 pairs). * p<0.01, ** p<0.001 vs. saline controls.

Results

Since the analysis of the number of occurrence of the particular type of behavior yielded highly similar results with the results showing the changes in the time of duration, only data of duration are reported in the graphs.

Morphine

Social interaction: All doses of MOR decreased the time spent by SI in total relative to saline controls [$F(3, 29)=7.76$; $p<0.001$] (Fig. 1). When analyzing particular patterns (Fig. 2), our data showed that all doses of MOR decreased the time [$F(3, 29)=9.16$; $p<0.001$] as well as number [$F(3, 29)=6.74$; $p<0.05$] of mutual sniffing and the time [$F(3, 29)=9.10$; $p<0.001$] as well as number [$F(3, 29)=9.60$; $p<0.001$] of allo-grooming. Only the highest dose (5 mg/kg) of MOR decreased the time spent by following [$F(3, 29)=3.55$; $p<0.05$] and the number of following [$F(3, 29)=4.44$; $p<0.05$]. In contrast, the time spent by [$F(3, 29)=11.49$; $p<0.0001$] and the number [$F(3, 29)=12.31$; $p<0.0001$] of genital investigation was increased by the dose of 5 mg/kg of MOR. Climbing over and crawling under was not affected by MOR.

Non-social activities: As shown in Figure 1, MOR did not affect locomotion, but two lower doses of MOR (1.0 and 2.5 mg/kg) increased the time spent by [$F(3, 29)=5.77$; $p<0.01$] and the number [$F(3, 29)=4.17$; $p<0.05$] of rearing when compared to saline controls.

THC

There were no significant differences in any measure between THC and ethanol groups. Therefore we added also control group administered with saline and run an additional statistical analysis.

Social interaction: All groups of THC as well as ethanol decreased the time spent by SI in total relative to saline controls [$F(4, 35)=9.26$; $p<0.0001$] (Fig. 3). When analyzing particular patterns (Fig. 4), our data showed that all doses of THC as well as ethanol decreased the time [$F(4, 35)=12.79$; $p<0.0001$] as well as number [$F(4, 35)=9.93$; $p<0.0001$] of mutual sniffing and the time [$F(4, 35)=10.92$; $p<0.0001$] as well as number [$F(4, 35)=13.12$; $p<0.0001$] of allo-grooming. There were no differences in any other SI patterns (following, genital investigation, climbing over and crawling under).

Non-social activities: Neither THC, nor ethanol affected locomotion of the animals. On the other hand, the time spent by [$F(4, 35)=10.2$; $p<0.0001$] and the

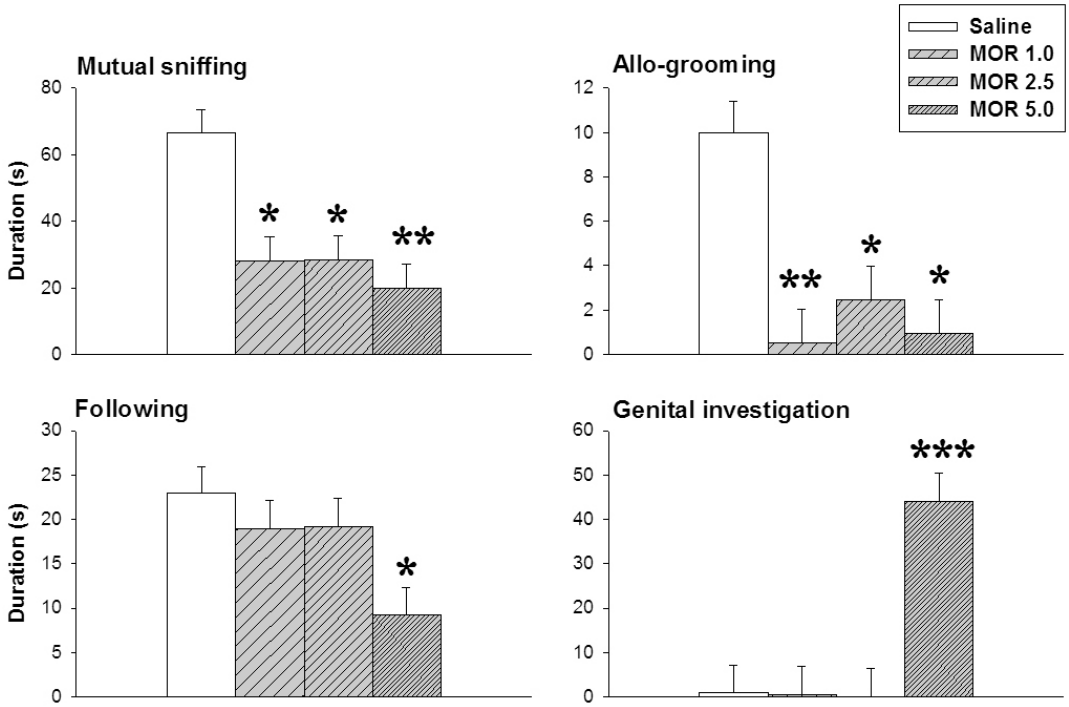

Fig. 2. The effect of morphine on particular patterns of social interaction in adult male rats. Values are mean ± SEM (n=8-9 pairs). * p<0.01, ** p<0.001 vs. saline controls.

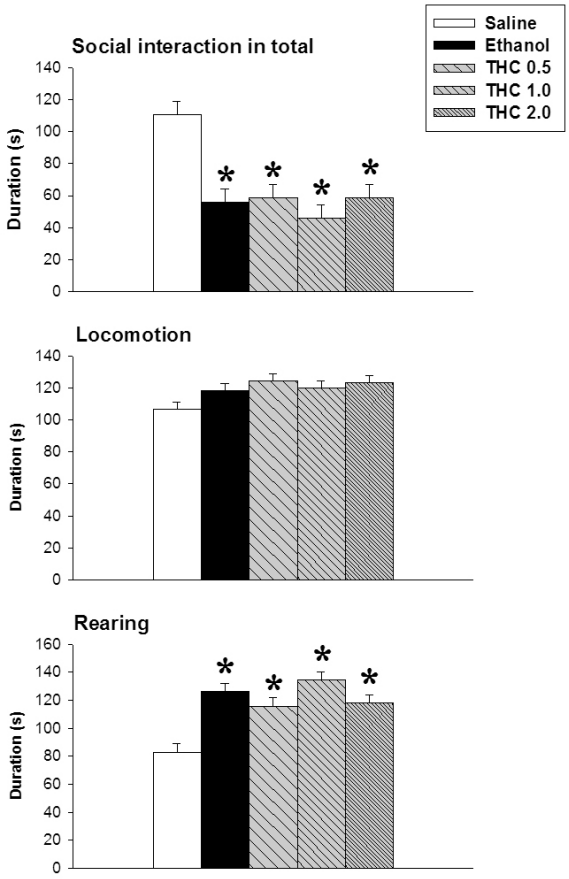

Fig. 3. The effect of THC and ethanol on social interaction in total and locomotor and exploratory behavior of adult male rats. Values are mean ± SEM (n=8-9 pairs). * p<0.01 vs. saline controls.

number [F(4, 35)=8.94; p<0.0001] of rearing was increased after all doses of THC as well as after ethanol relative to saline controls.

Discussion

The present study demonstrates that both, MOR and ethanol solution of THC, decreased the SI and increased the vertical exploratory behavior, when compared to control group administered with saline. Although, there were no differences in changes of behavior of animals which received THC, when compared to their proper control group – group with ethanol treatment. The explanation for such finding may be several. First, it is possible that the doses of THC (0.5; 1.0; 2.0 mg/kg) are too low to affect SI itself, excluding effect of ethanol. However, study of Cheer *et al.* (2000) showing development of positive place preference conditioning by using dose as low as 1.5 mg/kg of THC disproves this speculation. If such as low dose is able to induce active drug-seeking, which is a sign for drug addiction, it should be enough for affecting also other types of behavior. Second, it is possible that the effect of ethanol, even in low concentration 7.2 % overshadows the effect of THC *per se* and decreases SI. However, this hypothesis is not supported by studies of Varlinskaya and Spear (2009, 2010) showing that ethanol in concentration

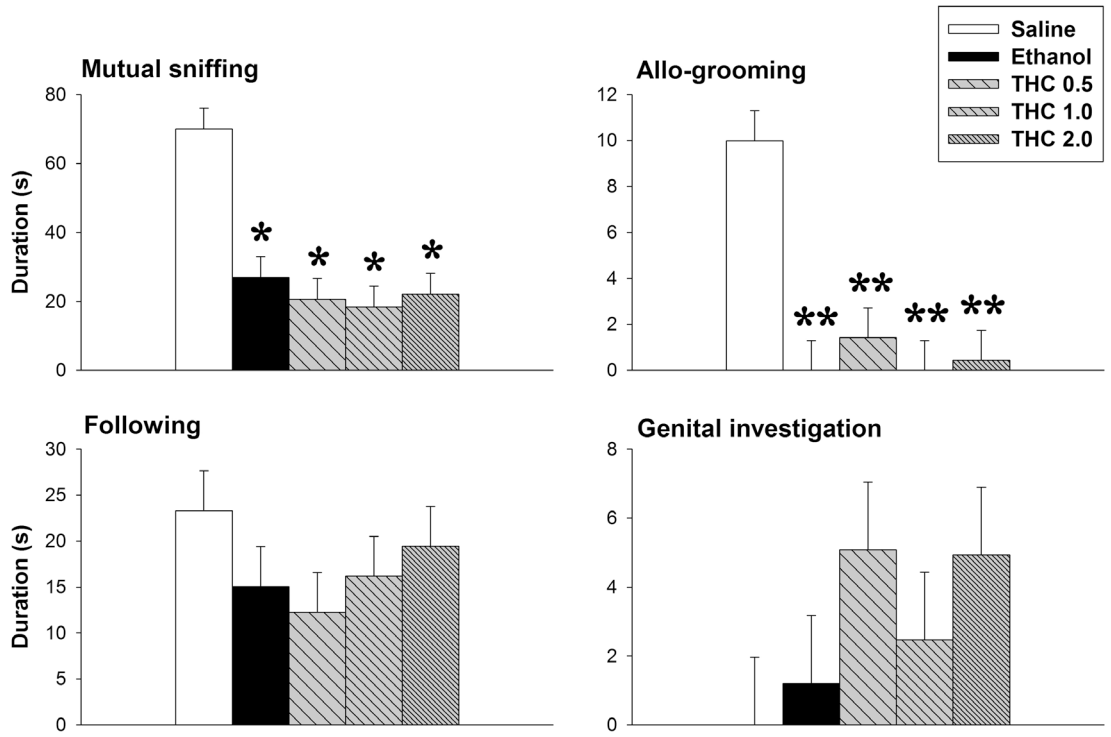

Fig. 4. The effect of THC and ethanol on particular patterns of social interaction in adult male rats. Values are mean ± SEM (n=8-9 pairs). * p<0.001, ** p<0.0001 vs. saline controls.

12.6 % injected in a dose of 0.5 and 0.75 g/kg was pro-socializing. Nevertheless, Trezza *et al.* (2009, 2014) suggested biphasic effect of ethanol; pro-social in lower doses and anti-social in higher doses. However, this is still in contradiction with our results. Third, it is also possible that the reason for the differences seen in the present study are not the effects of the drugs or ethanol, but the effect of saline. Saline administration was repeatedly shown to act as a stressor stimulus when compared to naïve controls without injection (Šlamberová *et al.* 2010a, Yamamotová *et al.* 2011). *In utero* daily saline injections may be considered as prenatal stressor that induces behavioral changes in adult progeny (Peters 1982, Schindler *et al.* 2004). Moreover, study of Kiyatkin and Lenoir (2011) showed that saline injection is able to produce significant changes in the CNS that may induce desynchronization in EEG rhythm. Thus, one may speculate that not the drugs and ethanol, but the saline injection is responsible for the differences found in the present study. However, this is just a speculation that would need to be verified in future studies that should compare the effect of saline injection with the sham control.

Our results show that MOR treatment decreases both, the time spent in SI as well as the occurrence of SI. Our data contradict the finding of Panksepp *et al.* (1980) showing that MOR at doses 1 mg/kg or lower reduced social separation-induced distress vocalizations in pups, decreased the proximity maintenance time in socially housed animals, increased social play and decreased maternal aggression. Also the study of Niesink *et al.* (1996) showed that MOR (10 mg/kg) exposure *in utero* was shown to increase social play in adolescent rodents and increase appetite, while decreasing effectiveness of sexual behavior (Vathy 1993). On the other hand, the present data are supported by our previous findings demonstrating that MOR (10 mg/kg) administered during the second half of pregnancy attenuates some components of maternal behavior, while increasing other "non-maternal" activities of mothers (Šlamberová *et al.* 2001). Other our study (Šlamberová *et al.* 2003) investigating maternal behavior showed that MOR (10 mg/kg) exposure *in utero* makes rats better mothers than controls, later on in adulthood. Thus, it seems that not only the dose, but also the timing of MOR exposure (*in utero* or in adulthood), may diametrically different impact the effect of MOR on social behavior.

All the above mentioned studies tested total SI only, without distinguishing between different social activities as the present study did. In the present set of experiments, detailed evaluation of SI was targeted to specific social behavioral patterns. Specifically, the

present study demonstrated that mutual sniffing as well as allo-grooming was decreased by all doses of MOR and ethanol/THC. The following of one animal by another was decreased only by the highest dose of MOR, but was not affected by ethanol or THC. The most interestingly, there was high increase of genital investigation after the highest dose (5 mg/kg) of MOR. Because genital investigation may be considered offensive aggression (Pellis 1988), it is possible that MOR in a dose of 5 mg/kg can induce aggression. This hypothesis is supported by several findings: MOR had been shown to produce aversion to the odor of pups (Kinsley *et al.* 1995), increase maternal aggression towards the pups (Haney and Miczek 1989, Rosenblatt *et al.* 1988) and to increase aggression in a group of adult male rats resulting in destructive and fatal fights (Kanui and Hole 1990). Because in our experimental settings we did not observe any forms of aggressive behaviors such as boxing, aggressive fighting or biting attack, that usually occur in other types of animal modes (e.g. in resident-intruder test), more studies using models of aggression will be necessary to confirm this hypothesis.

As for non-social activities, the present study did not find any differences induced by MOR or THC on locomotion, while there was an increase in vertical exploration (rearing) induced by lower doses of MOR (1.0 and 2.5 mg/kg) and by ethanol. Non-social activities were not affected by THC treatment when compared to ethanol controls. These data contradict findings showing that THC decreases locomotor activity (Hernandez-Tristan *et al.* 2000, Schramm-Sapyta *et al.* 2007). We suggest that the discrepancy might have been caused by a different dose of THC used in our study (0.5; 1.0; 2.0 mg/kg) and the study of Schramm-Sapyta (2007), which was high as 5 mg/kg. This idea is supported by study of Katsidoni *et al.* (2013) showing dose-dependent effect of THC on locomotion and rearing. Similarly, MOR seems to have dose-dependent effect: while higher doses of MOR (5; 10; 15; 20 mg/kg) were shown to sedate the animals and decrease their rearing activity (Patti *et al.* 2005, Šlamberová *et al.* 2012), lower doses of MOR increase it (Giorgi *et al.* 1997). The most surprising result of the present study was again the effect of ethanol. Studies of Varlinskaya and Spear (2009, 2010) showed that ethanol may lower locomotion, however only in a dose of 1.25 g/kg (concentration 12.6 %) but not lower. Similarly also Garcia-Cabrera and Berge (1988) demonstrated that ethanol in a dose of 1.5 g/kg depresses spontaneous behavior in rats. This dose is higher than the dose that was used in the present study. In addition, study of Duncan *et al.* (2000) showed that ethanol in doses of 800 or 1600 mg/kg increases rearing activity, which is in agreement with the present study. Therefore, also the effect of ethanol on spontaneous behavior seems to be dose-dependent. It is also suggested that the effect of ethanol may be due to the influence on dopaminergic receptors (Rubinstein *et al.* 1997, Sobrian *et al.* 2005). Another reason for the differences in locomotion seen in the present study and in the studies of others might be also different method of measure. While the present study measured locomotion as number of occurrence and time spent by the activity, studies of others usually use number of line crossing or walked distance (Hernandez-Tristan *et al.* 2000, Schramm-Sapyta *et al.* 2007, Varlinskaya and Spear 2009, Varlinskaya and Spear 2010).

In conclusion, based on the present results we can assume that opioids affect SI in greater extent than cannabinoid. The tested low doses of cannabinoid did not affect evaluated parameters from SIT. This outcome could be the result of ethanol masking an effect of THC. Future studies will examine the effect of ethanol, saline and sham injection on rat behavior.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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