Effect of kappa-opioid receptor agonists U69593, U50488H, spiradoline and salvinorin A on cocaine-induced drug-seeking in rats

Aashish S. Morani[1], **Bronwyn Kivell**[1], **Thomas E. Prisinzano**[2], and **Susan Schenk**[3]
Aashish S. Morani: aashish.morani@vuw.ac.nz; Bronwyn Kivell: Bronwyn.kivell@vuw.ac.nz; Thomas E. Prisinzano: prisinza@ku.edu; Susan Schenk: Susan.schenk@vuw.ac.nz

[1] School of Biological Sciences, Victoria University of Wellington, P.O.Box 600, Wellington, New Zealand [2] Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 6604, USA [3] School of Psychology, Victoria University of Wellington, P.O.Box 600, Wellington, New Zealand

Abstract

Our previous work indicated that pretreatment with the selective kappa opioid receptor (KOPr) agonist, U69593, attenuated the ability of priming injections of cocaine to reinstate extinguished cocaine-seeking behavior. The present study expanded these initial tests to include other traditional KOPr agonists, U50488H, spiradoline (SPR), and salvinorin A (Sal A), an active constituent of the plant *Salvia divinorum*. Following acquisition and stabilization of cocaine self-administration, cocaine-produced drug-seeking was measured. This test was conducted in a single day and comprised an initial phase of self-administration, followed by a phase of extinguished responding. The final phase examined reinstatement of extinguished cocaine self-administration followed by a priming injection of cocaine (20.0 mg/kg, intraperitoneal (I.P.)) in combination with the various KOPr agonists. Cocaine-induced drug-seeking was attenuated by pretreatment with U69593 (0.3 mg/kg, subcutaneous (S.C.)), U50488H (30.0 mg/kg, I.P.), SPR (1.0, 3.0 mg/kg, I.P.) and Sal A (0.3, 1.0 mg/kg, I.P.). Sal A (0.3, 1.0 mg/kg, I.P.) had no effect on operant responding to obtain sucrose reinforcement or on cocaine induced hyperactivity. These findings show that Sal A, like other traditional KOPr agonists attenuates cocaine-induced drug seeking behavior.

Keywords

1. Introduction

Prior studies have demonstrated that KOPr agonists attenuate some of the neuro-chemical and behavioral effects of drugs of abuse (Heidbreder et al., 1993Heidbreder et al., 1995; Shippenberg et al., 1996; Thompson et al., 2000). These effects might be due to the interactions between KOPr and dopaminergic systems since pretreatment with kappa-opioid agonists decreased dopamine (DA) concentrations in terminal regions (Devine et al., 1993;

Corresponding Author: Susan Schenk, School of Psychology, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand. Tel: +64–4–463–6034; Fax: +64–4–463–5402. Susan.schenk@vuw.ac.nz.

Shippenberg et al., 1994; Maisonneuve et al., 1994; Heidbreder et al., 1994, 1996) and attenuated cocaine- (Shippenberg et al., 1994), amphetamine- (Gray et al., 1999) and heroin- (Xi et al., 1998) induced DA release in the ventral striatum. Also, many behavioral effects of psycho-stimulants like cocaine (Heidbreder et al., 1993Heidbreder et al., 1995), amphetamine (Gray et al., 1999) and nicotine (Hahn et al., 2000) were decreased by prior administration of KOPr agonists. Additionally, pre-treatment with KOPr activating compounds attenuated cocaine (Glick et al., 1995; Mello and Negus, 1996; 1998; Negus et al., 1997; Schenk et al., 1999b; 2001), morphine (Glick et al., 1995) and heroin (Xi et al., 1998) self-administration in laboratory animals. These findings suggest the possible role of KOPr agonists as pharmacotherapeutics in the treatment of addiction (Mello and Negus, 1998; Prisinzano et al., 2005; Shippenberg et al., 2007; Willmore-Fordham et al., 2007; Prisinzano and Rothman, 2008; Tomasiewicz et al., 2008).

During a binge of cocaine self-administration in abusers there is tolerance to many of the subjective effects (Fischman et al., 1985). Self-administration and drug craving, however, continue relatively unabated. Since exposure to cocaine produces craving for more cocaine (Jaffe et al., 1989), it is possible that continued exposure to cocaine during a binge maintains a cycle of drug-seeking and drug-taking, even after tolerance develops. If so, identification of factors that reduce the ability of cocaine to produce drug-seeking would be an important step in the development of effective treatments for cocaine abuse.

Animal models of drug-seeking have contributed greatly to investigations of these factors. A number of laboratories have used procedures developed by de Wit and Stewart (1981) to demonstrate the ability of cocaine to reinstate extinguished cocaine self-administration behavior. This effect appears to be mediated, at least in part, by dopaminergic mechanisms since cocaine-produced drug-seeking was attenuated by pre-treatment with dopaminergic agonists and antagonists (Self et al., 1996; Khroyan et al., 2000; 2003; Alleweireldt et al., 2002).

Pretreatment with the kappa-opioid receptor agonist, U69593 attenuated cocaine-produced reinstatement of extinguished cocaine-taking behavior (Schenk et al., 1999b; 2000). This effect was somewhat specific since reinstatement produced by experimenter-administered injections of the dopamine uptake inhibitors, GBR 12909 or WIN 35428, was not attenuated by pretreatment with U69593 (Schenk et al., 2000). The attenuation of drug-seeking involved effects at central KOPr's, as prior intracerebroventricular administration of nor-BNI, a kappa-opioid antagonist, reversed the effects of U69593 (Schenk et al., 1999b).

Sal A, a neoclerodane diterpene, is an active constituent of the hallucinogenic sage, *Salvia divinorum,* and has been used in traditional, spiritual and ethnopharmacological practices by the Mazatec Indians of Oaxaca, Mexico (Valdes et al., 1983, Valdes 1994; Siebert 1994). Sal A binds selectively to the KOPr (Roth et al., 2002; Yan and Roth, 2004; Prisinzano 2005) with greater efficacy than U69593 and U50488H (Chavkin et al., 2004). It has a rapid onset of action and a short elimination half life (56.6 ± 24.8 min) (Schmidt et al., 2005; Butelman et al., 2009). Accordingly, there has been recent interest in understanding the neuropharmacology of Sal A and comparing it with traditional KOPr agonists.

Sal A dose dependently produced antinociception in tail flick, hot plate and acetic acid induced writhing in mice that was antagonized by nor-BNI (McCurdy et al., 2006; John et al., 2006). Sal A also produced discriminative stimulus effect in rats (Willmore-Fordham et al., 2007; Baker et al., 2009) and rhesus monkey's (Butelman et al., 2004), decreased mobility in the forced swim test (Carlezon et al., 2006) and produced motor inco-ordination in mice (Fantegrossi et al., 2005; Zhang et al., 2005). The aim of our current study was to

determine whether Sal A has similar effects to other KOPr agonists, U69593, U50488H and SPR on cocaine produced drug-seeking in rats.

2. METHODS

2.1. Subjects

Male Sprague-Dawley rats weighing 325–350 g were used. They were housed individually in hanging polycarbonate cages. The humidity (55%) and temperature (19–21°C) were controlled and food and water were freely available except during testing. Animals tested for sucrose self-administration had free access to water all the times within the home cage and were maintained at approximately 85% of their initial feeding weight during the experiments by restricting access to food. The animal colony was maintained in the animal facility at School of Psychology, Victoria University of Wellington. All the experiments were conducted in accordance with the guidelines of the Animal Ethics Committee of Victoria University of Wellington. Lights were maintained on a 12:12 hr cycle with lights on at 0700.

2.2. Surgery

Under deep anesthesia produced by ketamine/xylacine (90/9 mg/kg, I.P.), a silastic catheter was placed in the right jugular vein. The external jugular vein was isolated, the catheter was inserted and the distal end (22 gauge stainless steel tubing) was passed subcutaneously to an exposed portion of the skull where it was fixed to embedded jewele's screws with dental acrylic.

Each day, the catheters were infused with 0.1 ml of a sterile saline solution containing heparin (30.0 U/ml), penicillin G Potassium (250,000 U/ml) and streptokinase (8000 U/ml) to prevent infection and the formation of clots and fibroids. The rats were allowed five days post surgery for recovery.

2.3. Apparatus

2.3.1. Cocaine Self administration—Self-administration testing was carried out in a humidity (55%) and temperature (19–21°C) controlled environment in standard operant chambers (Med Associates, ENV-001) equipped with 2 levers. Depression of one lever (the active lever) resulted in a 0.1 ml intravenous infusion of cocaine HCl dissolved in sterile physiological saline containing heparin (3.0 U/ml). Infusions were of 12 s duration. Coincident with drug delivery was the illumination of a stimulus light located directly above the active lever. This stimulus light remained illuminated throughout each 12 s infusion. Depression of the other lever (the inactive lever) was without programmed consequence.

Rats were maintained in the animal colony except during testing. Immediately prior to each daily test, the catheter lines were infused with 0.1 ml of the heparin-penicillin-streptokinase solution. The stainless steel catheter was connected to a length of microbore tubing and connected to the syringe. At the end of each test, the lines were again infused with 0.1 ml of the heparin-penicillin-streptokinase solution, the stainless steel tubing was plugged and the animal was returned to its home cage. Drug delivery and data acquisition were controlled by Med Associates software. Cocaine deliveries were made via mechanical pumps (Razel, Model A with 1.0 rpm motor equipped with 20 ml syringe).

2.3.2. Sucrose self-administration—Training and testing procedures were conducted in eight standard operant chambers (Med Associates, ENV001) in an unlit, sound attenuating room. Operant chambers were quipped with two retractable levers and a sucrose bottle delivering 0.1 ml of 10% sucrose solution onto a tray on the chamber wall according to the imposed schedule of reinforcement. Sucrose delivery and data acquisition were

controlled by Med Associates software. Experiments were conducted between 0900 and 1600 hours.

2.3.3. Locomotor activity tests—Eight Open field chambers (Med Associates) equipped with two banks of sixteen photocells on each wall were used to measure horizontal locomotion. The open field boxes were interfaced with a microcomputer located in an adjacent laboratory. Testing was conducted in the dark between 1000 and 1600 hours. White noise was continually present to mask extraneous disturbances.

2.4. Procedure

2.4.1 Cocaine Self administration training—Acquisition of cocaine self-administration was monitored during daily 2 hr sessions. Each session began with an experimenter-delivered infusion of cocaine (0.5 mg/kg/infusion). Thereafter, depression of the active lever produced automated cocaine infusions according to an FR-1 schedule of reinforcement. The criterion for acquisition of cocaine self-administration consisted of at least 20 reinforced responses (10 mg/kg) during the 2 hr session and a ratio of active: inactive lever responses of at least 2:1. Self-administration was considered acquired when these criteria were met for three consecutive days. Following acquisition, the response requirements were increased to FR-5. Daily two hr sessions were conducted until there was less than 20% variation in responding on three consecutive days. During training, the cocaine infusion was always paired with the illumination of a house light located directly above the active lever.

2.4.2. Cocaine reinstatement test—Once responding on the FR-5 schedule was stable, the effect of prior administration of the kappa-opioid agonists, U69593, U50488H, SPR and Sal A on drug-seeking produced by cocaine was measured. As with our previous studies (Schenk et al., 1999b; 2000), this test was conducted in a single day and consisted of three phases. The first phase was comprised of a one hr period of cocaine self-administration (0.5 mg/kg/infusion, FR-5 schedule of reinforcement) in which the light stimulus was paired with cocaine infusions. After the one hr self-administration period, the cocaine solution was replaced with heperanized saline and responding was reinforced with this vehicle infusion (FR-5 schedule of reinforcement) and illumination of the light stimulus. Responding during this phase was measured for 3 hrs. At the start of the third phase during which responding was again reinforced according to an FR-5 schedule of reinforcement with an infusion of vehicle solution and illumination of the light stimulus, separate groups of rats (n=5–7 per group) received an injection of U69593 (0.03, 0.1 or 0.3 mg/kg, S.C.), U50488H (3.0, 10.0 or 30.0 mg/kg, I.P.), SPR (0.3, 1.0 or 3.0 mg/kg, I.P.), Sal A (0.1, 0.3 and 1 mg/kg, I.P.) or vehicle. These injections were administered either 5 (Sal A), 15 (U69593) or 30 (U50488H, SPR) min prior to an injection of cocaine HCl (20 mg/kg, I.P.). Responding was measured for 60 min following the cocaine injection.

2.4.3. Sucrose reinforcement training and test—Animals were trained to self-administer sucrose using an auto-shaping procedure. Training sessions were 45 min duration on each of 10 training days. Once stable responding was produced, the animals were maintained on an FR1 schedule of reinforcement during which depression of the active lever (left lever) delivered 0.1 ml of a 10% sucrose solution. Following acquisition, the response requirements were increased to FR-5. Daily one hr sessions were conducted until there was less than 20% variation in responding for three consecutive days. Once responding on the FR-5 schedule was stable, the effect of Sal A on sucrose-reinforced responding was measured. Rats received an injection of either Sal A (0.3 or 1.0 mg/kg, I.P.) or vehicle 5 min prior to sucrose self-administration testing and the number of responses was measured for 60 min.

2.4.4. Procedure for cocaine-produced locomotor activity—On the test day, separate groups of rats (n=6 per group) received an injection of Sal A (0.0 or 0.3 mg/kg, I.P.) 5 minutes prior to an injection of cocaine (20 mg/kg, I.P.). Immediately following the second injection, the rats were placed in the activity chambers and total activity, a compilation of horizontal and vertical activity, was measured for a period of 60 min.

2.5. Data Analysis

The number of responses produced during the one-hour period following the cocaine injection (20 mg/kg, I.P.) at the start of phase 3 was measured. Sucrose-reinforced responding was measured for a one-hour period following Sal A or vehicle exposure. Statistical analysis for cocaine-induced reinstatement and sucrose reinforcement consisted of one-way ANOVAs followed by Tukey post-hoc comparisons where appropriate. Student t-test was applied for locomotion test.

2.6. Drugs

Cocaine HCl (Merck Pharmaceuticals, Palmerston North, New Zealand), SPR and U50488H (Sigma-Aldrich, St. Louis, MO) were dissolved in physiological saline. U69593 (National Institute of Drug Abuse) was dissolved in an aqueous solution of 25% propylene glycol. Sal A (isolated by Dr. Thomas E. Prisinzano, University of Kansas, Lawrence, KS) was suspended in 75% DMSO. Sucrose was dissolved in tap water. Intravenous infusions were in a volume of 100 μl and S.C. or I.P. injections were in a volume of 1 ml/kg. All drug weights refer to the salt.

3. Results

Figure 1 shows the responding during phases 1 and 2 for a representative group of rats from the present study. During the 1 hr period of cocaine self-administration, responding was high. When saline was substituted for cocaine during phase 2, responding decreased to less than 20 responses during hr 3.

Figure 2 shows the number of saline-reinforced responses produced during the 1 hr period following the injection of cocaine at the start of Phase 3 for rats that received systemic administration of the KOPr agonists, U69593, U50488H, SPR and SalA. Each of the KOPr agonists produced a dose-dependent reduction of cocaine-produced reinstatement (U50488H: $F_{(3,17)}=4.277$, $p<0.05$; U69593: $F_{(3,20)}=3.103$, $p<0.05$); SPR: $F_{(3,21)}=7.899$, $p<0.01$; Sal A: $F_{(3,23)}=79.33$, $p<0.0001$). Doses of 30.0 mg/kg U50488H, 0.3 mg/kg U69593, 1.0 or 3.0 mg/kg SPR and 0.3 or 1.0 mg/kg Sal A significantly decreased drug-seeking ($p<0.05$).

Figure 3 shows the number of sucrose-reinforced responses produced during the 1 hr period following the injection of Sal A (0.3 and 1.0 mg/kg, I.P.). Sal A did not significantly decrease sucrose self-administration ($F_{(2,27)}=0.06$, $p=0.942$).

Figure 4 shows the effect of Sal A (0.0 or 0.3 mg/kg, I.P.) on cocaine-produced locomotor activity. Sal A failed to decrease cocaine-produced hyperactivity. ($p=0.85$).

4. Discussion

As has previously been shown, cocaine reinstated extinguished cocaine-taking behavior (Worley et al., 1994; Schenk and Partridge 1999a; Schenk et al., 1999b). This effect was attenuated by pretreatment with KOPr agonists. High doses of SPR might have produced a non-selective effect on motor behavior as suggested by the decreased responding observed in a drug discrimination study (Holtzman et al., 1991). The attenuation of drug-seeking was

also produced by a dose of SPR (1.0 mg/kg) that did not decrease responding in a drug discrimination task (Holtzman 2000). It is therefore unlikely that the decrease in cocaine-seeking represents a generalized inability to perform the lever press operant at the dose used in this study. Similarly, decreased drug-seeking was produced by doses of U69593 and U50488H that failed to produce a generalized decrease in motor activity (Schenk et al., 1999b; unpublished findings).

In a previous study, U50488H administered to mice 60 min prior to cocaine potentiated cocaine-induced conditioned place preference (CPP) but when administered 15 min prior to cocaine it suppressed the cocaine-CPP (McLaughlin et al. 2006). In this study we measured cocaine-induced drug-seeking during a 60 min period following U50488H administration and also observed a small but non-significant increase in responding.

In drug discrimination tests, Sal A and its synthetic derivatives substituted completely for U69593 (Baker et al., 2009), suggesting an effect mediated by the KOPr. Because of the interaction between KOPr and dopaminergic mechanisms, a focus on the effects of Sal A on dopamine-mediated behaviors has been of interest. Cocaine-seeking has been attributed to dopaminergic mechanisms and there are data to support the idea that Sal A modulates the effects of cocaine through interactions with D1 receptor mediated signaling in the dorsal striatum (Chartoff et al., 2008; Gehrke et al., 2008). Low doses of Sal A (40 μg/kg) increased DA levels in nucleus accumbens (NAc) (Braida et al., 2008) whereas higher doses decreased NAc DA levels (Zhang et. al., 2005). Thus, the ability of moderate to high doses of Sal A to decrease cocaine-produced drug-seeking might be due to its effects on the dopaminergic system.

Alternatively, the decrease in drug-seeking might reflect a non specific effect. This possibility was tested by examining the effects of Sal A on responding maintained by a sucrose reinforcer. Conditions for this test were comparable to conditions of the reinstatement tests. In this experiment, responding maintained by sucrose was high and comparable to responding maintained by cocaine. Doses of Sal A (0.3, 1.0 mg/kg I.P.) that decreased cocaine-seeking failed to alter sucrose reinforced responding.

Additionally, KOPr activation has shown to produce motor inco-ordination (Fantegrossi et al., 2005). A more recent report, however, failed to find any effect of Sal A on locomotor activity (Baker et al., 2009). The effect of Sal A on cocaine-produced hyperactivity was measured in the present study under conditions that were comparable to the conditions of the reinstatement tests. Sal A did not attenuate cocaine-produced hyperactivity. Therefore, the decrease in drug-seeking produced by Sal A cannot be attributed to either a generalized inability to perform the lever press operant or a disruption of motor activity.

Reinstatement of conditioned place preference (CPP) following extinction has also been used as a model of drug-seeking (Kreibich and Blendy, 2004) and a modulation of drug-seeking behaviors by traditional KOPr agonists has been demonstrated using this model. The effects appear, however, to be opposite to those produced when drug-seeking in a self-administration paradigm are measured. For example, foot-shock or forced swim stress were effective in reinstating both extinguished place preference (McLaughlin et al., 2003; 2006; Redila and Chavkin, 2008) and self-administration (Beardsley et al., 2005). The effect in the CPP paradigm was, however, blocked by pretreatment with the KOPr antagonists, nor-BNI (Redila and Chavkin, 2008; McLaughlin et al., 2003). The effect of KOPr antagonists might depend on the drug-seeking stimulus since the KOPr antagonist, JDTic inhibited stress induced drug seeking but had no effect on cocaine-produced reinstatement (Beardsley et al., 2005).

Further, KOPr knock out mice were insensitive to stress-produced reinstatement, as measured in the CPP paradigm, supporting the idea that reinstatement of CPP required KOPr activation. A further difference in the role of KOPr in reinstatement as measured in the CPP and self-administration paradigms is that U50488H potentiated the cocaine-produced place preference whereas it decreased drug-seeking following extinction of self-administration (present study). Additional studies will have to be conducted in order to tease apart the different aspects of the two paradigms.

In conclusion, Sal A, like traditional KOPr agonists attenuated cocaine-induced cocaine seeking. Further studies on Sal A and its structural derivatives will help us understand the mechanism by which KOPr agonists attenuate drug-seeking behaviors and to determine weather Sal A produces the same magnitude of adverse effects that have limited the development of other KOPr agonists as anti-addiction pharmacotherapies.

Acknowledgments

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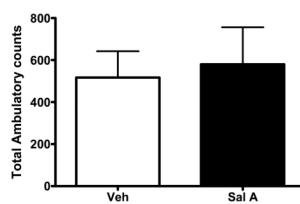
Fig. 1.
Effect of salvinorin A (Sal A) on spontaneous locomotion. Total activity (+SEM) recorded for 60 min following vehicle (Veh) and Sal A (0.3 mg/kg) pre-treatment. Student t-test. N= 7 for each group.
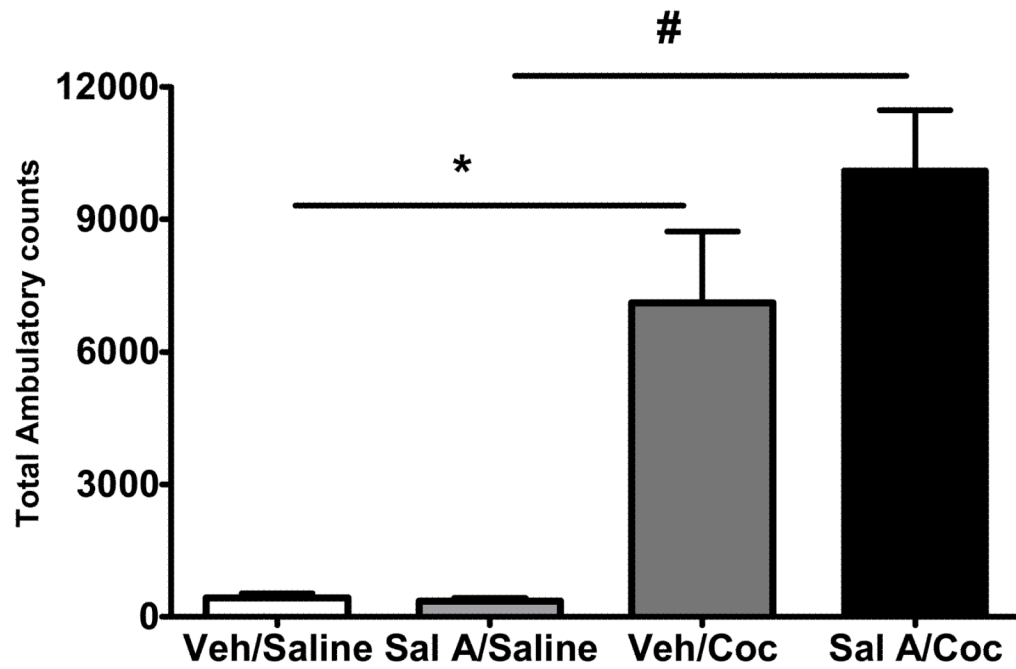
Fig. 2.
Effect of salvinorin A (Sal A) on cocaine induced hyperactivity. Drug naive rats were initially injected with vehicle (Veh, 75% DMSO) or Sal A (0.3 mg/kg) followed by saline (1 ml/kg) or cocaine (Coc, 20 mg/kg) and total activity was monitored for 60 min. Data expressed as mean total activity (+SEM). $*p<0.05$, data compared with Veh/Saline treated group, $\#p<0.05$, data compared with Sal A/Saline treated group. One way ANOVA followed by Tukey test. n= 6–7 for each group.
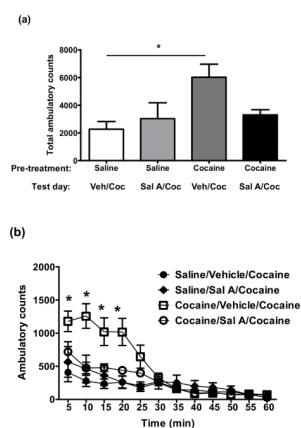
Fig. 3.
Effect of salvinorin A (Sal A) on expression of cocaine sensitization. Rats were injected with saline (1 ml/kg) or cocaine (20 mg/kg) for 5 consecutive days. Animals remained drug free from day 6–9. On day 10, rats were injected with either vehicle (Veh, 75% DMSO) or Sal A (0.3 mg/kg) and 5 min later were injected with cocaine (Coc, 20 mg/kg) and activity was measured for 60 min. (a) Data expressed as mean total activity (+SEM). $p < 0.05$, data compared with Saline/Vehicle/Cocaine treated group. One way ANOVA followed by Tukey test. (b) Time course measurement of mean ($\pm$SEM) of locomotion activity over a period of 5 min interval. $p < 0.05$, vs. Cocaine/Sal A/Cocaine treated group. Two way ANOVA followed by Bonferroni post hoc test. n= 6-8 for each group.
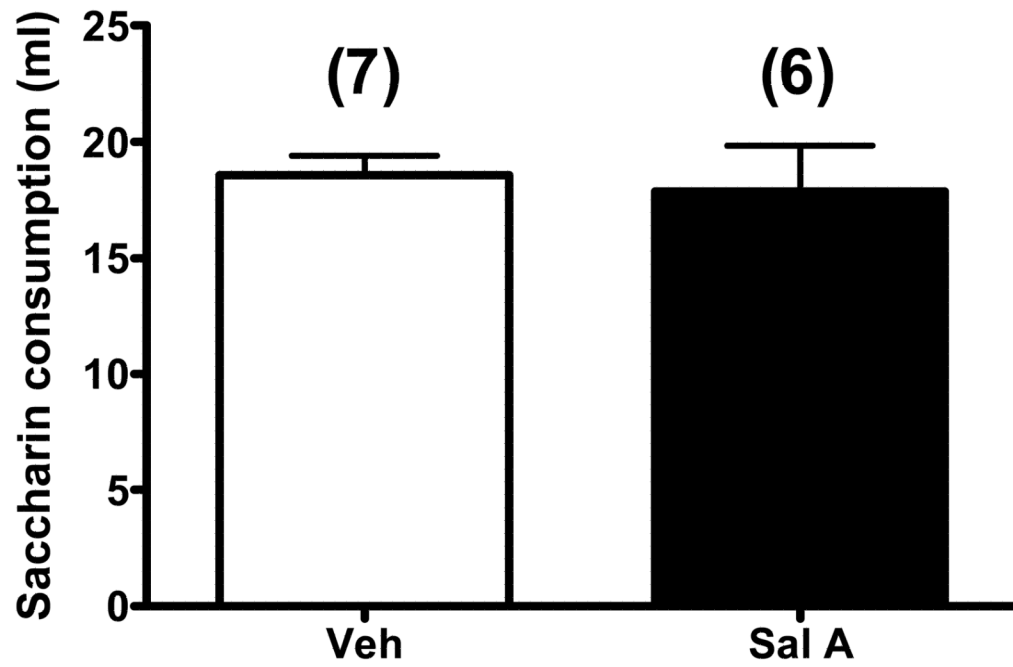
Fig. 4.
Effect of salvinorin A (Sal A) on conditioned taste aversion. Saccharin consumption in ml (+SEM) by rats treated with either vehicle (Veh) or Sal A (0.3 mg/kg) on test day. Student t-test. Numbers in parenthesis indicate sample size.
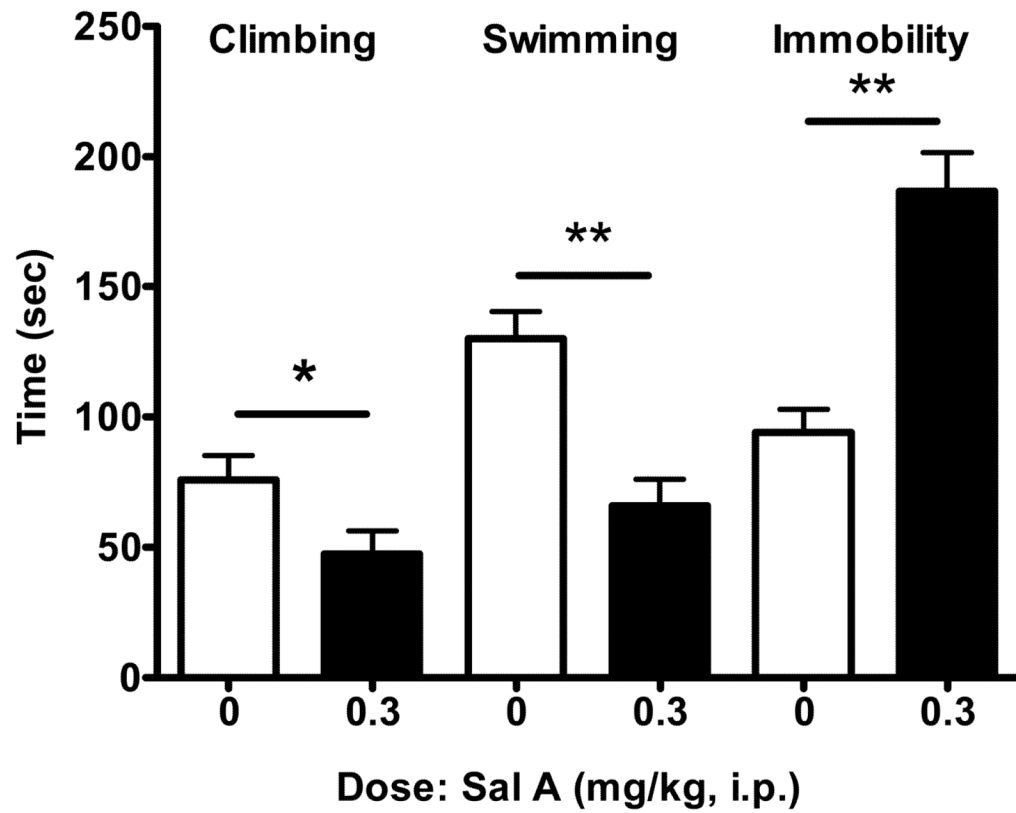
Fig. 5.
Effect of single injection of salvinorin A (Sal A) on forced swim test (FST) in drug naive rats (n=6). On test day, animals were injected with Sal A and 5 min later were subjected to FST. Data expressed as mean time (sec) (+SEM) for climbing, swimming and immobility behaviours during 5 min of FST. *p<0.05, **p<0.01. Data for 0.3 mg/kg compared with 0 mg/kg for climbing, swimming and immobility. Mann Whitney test. n= 6 for each group.

Table 1

Experimental groups for the effects of Sal A on cocaine induced hyperactivity.

Acute pre-treatment (ip)	Challenge injection (ip)
Veh	Saline (1 ml/kg)
Sal A	Saline (1 ml/kg)
Veh	Cocaine(20 mg/kg)
Sal A	Cocaine (20 mg/kg)

Veh, vehicle (75% DMSO); Sal A, Salvinorin A (0.3 mg/kg)

Table 2

Experimental groups for the effects of Sal A on expression of cocaine sensitization.

Pretreatment (day 1–5) (ip)	Acute treatment (day 10) (ip)	Challenge injection (day 10)(ip)
Saline (1 ml/kg)	Veh	Cocaine (20 mg/kg)
Saline (1 ml/kg)	Sal A	Cocaine (20 mg/kg)
Cocaine (20 mg/kg)	Veh	Cocaine (20 mg/kg)
Cocaine (20 mg/kg)	Sal A	Cocaine (20 mg/kg)

Veh, vehicle (75% DMSO); Sal A, Salvinorin A (0.3 mg/kg)