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Single injection of novel kappa opioid receptor agonist salvinorin A attenuates expression of cocaine induced behavioral sensitization in rats

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Abstract

Kappa opioid receptor (KOPr) activation antagonizes many cocaine-related behaviors but adverse side effects such as sedation, dysphoria and depression limit their therapeutic use. Recently, salvinorin A (Sal A), a naturally occurring KOPr agonist, has been shown to attenuate cocaine-induced drug-seeking in a model of relapse in rats. The present study evaluated the effects of acute Sal A exposure on cocaine-induced hyperactivity and cocaine sensitization in rats. Acute treatment with the dose of Sal A that decreased drug-seeking in a previous study (0.3 mg/kg), significantly attenuated the expression of cocaine sensitization. This dose of Sal A failed to affect spontaneous locomotion or to produce a conditioned taste aversion to a novel-tasting saccharin solution. However, Sal A decreased climbing and swimming time and increased time spent immobile in the forced swim test. These findings indicate that Sal A, just like traditional KOPr agonists, attenuates cocaine-induced behavioral sensitization but does not produce the adverse effect of conditioned aversion, suggesting improved potential compliance. However, pro-depressive effects were also produced and these effects may limit the therapeutic potential.

Keywords

Salvinorin A; kappa opioid agonist; behavioural sensitization; conditioned taste aversion; forced swim test; depression; rat

Introduction

Kappa opioid receptor (KOPr) agonists have previously been shown to antagonize several cocaine-induced behaviors such as self-administration (Glick *et al.*, 1995; Kuzmin *et al.*, 1997), reinstatement of drug-seeking (Schenk *et al.*, 1999; 2000, Morani *et al.*, 2009; Sun *et al.*, 2010), hyperactivity (Heidbreder *et al.*, 1993; Vanderschuren *et al.*, 2000; Collins *et al.*, 2001) and sensitization to conditioned rewarding effects (Shippenberg *et al.*, 1996; Heidbreder et al., 1995). These potential anti-addictive properties of this class of compounds have prompted studies aimed at developing KOPr ligands as anti-addiction pharmacotherapies (Mello and Negus, 2000; Prisinzano *et al.*, 2005; Shippenberg *et al.*,

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2007; Tomasiewcz *et al.*, 2008). However, adverse effects produced by traditional KOPr agonists (U504885, U69593, bremazocine, ethylketazocine, Mr2033) such as depression (Todtenkopf *et al.*, 2004; Mague *et al.*, 2003), aversion (Mucha and Herz, 1985; Shippenberg and Herz, 1986), sedation (Wadenberg, 2003; Mello and Negus, 2000) and dysphoria (Walsh *et al.*, 2001) have limited their clinical utility.

Salvia divinorum has been abused as a recreational hallucinogen, particularly among adolescents and young adults (Griffin *et al.*, 2008; Kelly, 2011). Although, salvia use is banned in some countries such as Australia, Italy, Denmark and Sweden, it is legally available in most parts of the United States and New Zealand (Vorthermes and Roth, 2006; Griffin *et al.*, 2008; Kelly, 2011). Recent studies show that Sal A, an active component of the plant *Salvia divinorum* is a potent and selective KOPr agonist (Roth *et al.*, 2002; Yan and Roth, 2004). Sal A has a rapid onset and short duration of action (Schmidt *et al.*, 2005; Hooker *et al.*, 2008; 2009; Butelman *et al.*, 2007). It has a unique structure but shares many pharmacological properties with traditional KOPr agonists, including antinociception (McCurdy *et al.*, 2006; John *et al.*, 2006), discriminative stimulus effects (Willmore-Fordham *et al.*, 2007; Baker *et al.*, 2009), sedation (Fantagoressi *et al.*, 2005; Zhang *et al.*, 2005) and depression (Carlezon *et al.*, 2006). Sal A has also been shown to dose-dependently and selectively attenuate cocaine-induced drug seeking (Morani *et al.*, 2009). These findings support the development of novel neoclerodane diterpene KOPr agonists as potential pharmacotherapies for cocaine dependence (Prevatt-Smith and Prisinzano, 2010). Despite these promising findings, few studies have described other behavioural effects of Sal A.

Previous studies have shown that the conditioned and locomotor behavioral response to cocaine becomes sensitized following either self-administered (Hooks *et al.*, 1994; Phillips and Di Ciano, 1996) or experimenter-administered (Shippenberg *et al.*, 1996; Shippenberg and Heidbreder, 1995; Heidbreder *et al.*, 1996) exposures. The mesocorticolimbic dopaminergic system has been implicated in cocaine-produced sensitization, as an increase in extracellular DA levels has been observed following cocaine exposure in the VTA (Kalivas and Duffy., 1993b; Reith *et al.* 1997) and NAc (Di Chiara and Imperato, 1988; Kalivas and Duffy., 1990; 1993a,b; Cadoni *et al.*, 2000). Development of behavioral sensitization is a paradigm that highlights the ability of cocaine to alter neural circuits underlying its psychomotor effects (Robinson and Berridge, 1993; 2001; 2003; Kalivas *et al.*, 1998; Vanderschuren and Pierce, 2010). Therefore, finding pharmacological interventions to counter cocaine sensitization in animals could be a useful tool in identifying anti-cocaine agents. To the best of our knowledge, no work has been reported on the effect of Sal A on cocaine induced behavioral sensitization. Therefore, one of the aims of the current study was to investigate the effects of acute systemic Sal A on cocaine locomotor sensitization in rats.

Traditional KOPr agonists have been shown to produce aversion (Mucha and Herz, 1985; Bals-Kubik *et al.*, 1993) and depression (Todtenkopf *et al.*, 2004) in animal models. Sal A produced place aversion and sedation in C57BL/6J mice (Zhang *et al.*, 2005). In zebrafish and Wistar rats, a low dose of Sal A produced place preference, whereas at a higher dose, Sal A produced place aversion (Braida *et al.*, 2007; 2008). Sal A produces hallucinations in humans (Valdes et *al.*, 1983; Valdes, 1994; Johnson *et al.*, 2011) and non-human primates (Butelman *et al.*, 2009) and causes motor suppression in mice (Zhang *et al.*, 2005; Fantagrossi *et al.*, 2005). However, the aversive and sedative effects of doses of Sal A that selectively attenuated cocaine seeking (0.3 mg/kg) unknown. Therefore, we evaluated the aversive effect of Sal A using a conditioned taste aversion (CTA) paradigm (Smith *et al.*, 1964; Fenu *et al.*, 2005) and also measured effects on spontaneous locomotor activity (Hooker *et al.*, 2009). Recent reports on the effects of Sal A on depression have been

equivocal with both pro- (Carlezon *et al.*, 2006) and anti-depressant effects (Braida *et al.*, 2009) reported. The forced swim test (FST) is a widely used behavioral method to measure a depression-like effect (Porsolt *et al.*, 1979; Carlezon *et al.*, 2006) that has been used to screen anti-depressant drugs in laboratory tests (Detke *et al*, 1995). Therefore, we also evaluated the effects of a single injection of Sal A (0.3 mg/kg) on swimming behaviors, using the FST in rats.

Methods

Subjects

Male Sprague-Dawley rats were bred in the *vivarium* at The School of Psychology, Victoria University. All animals (200-250 g) were housed individually in polycarbonate cages at least 5 days prior to the experiment at the animal facility under controlled temperature (20 ± 1°C) and humidity conditions (55% Relative Humidity). Lights were maintained at a 12:12 h, with lights on at 07.00 h. All rats used for the experiments were drug naive and were handled by the experimenter for at least 5 days prior to the commencement of experiments to avoid handling stress. For cocaine-induced locomotion tests, spontaneous open field activity and FST experiments, rats had free access to food and water except during testing. For the CTA experiments, rats were water deprived for 23 h during the habituation period and for 23 h 20 min during the saccharin sessions. Food was freely available. All experimental procedures were approved by the Animal Ethics Committee of Victoria University of Wellington.

Apparatus for locomotion tests

Eight open field chambers (Med Associates, ENV-520) equipped with two banks of sixteen photocells on each wall were used to measure horizontal locomotion. Interruption of 3 adjacent photobeams, equivalent to the size of the rat, defined one horizontal activity count. Stereotypic counts during the sensitization experiments were determined by measuring repetitive beam breaks obtained from the activity monitoring software (Med Associates). The open field boxes were interfaced with a microcomputer located adjacent to the boxes. Testing was conducted in the dark in the continuous presence of white noise. For all activity experiments, rats were initially habituated to the locomotion chamber for 30 min. The animals then received drug treatment and were immediately returned to the activity chamber for 60 min. All experiments were carried out between 10.00 and 17.00 h.

Procedure for spontaneous and cocaine-induced locomotion tests

Drug naïve rats were used (n=14 for spontaneous open field test and n=26 for cocaine-induced hyperactivity test). For spontaneous activity tests, separate groups of rats were injected on the test day with either vehicle (75% DMSO) or Sal A (0.3 mg/kg, i.p.) and locomotor activity was measured for 90 min (30 min habituation + 60 min post treatment). For the cocaine-induced activity test, animals were initially habituated in the activity chamber for 30 min. Following this, animals were randomly selected and injected with either vehicle (75% DMSO) or Sal A (0.3 mg/kg, i.p.). Five min after the first injection rats received either 0.9% saline or cocaine (20 mg/kg, i.p.) and ambulatory counts wer measured for 90 min (30 min habituation + 60 min post treatment).

Expression of cocaine sensitization and cocaine produced stereotypy

A total of 27 drug naïve rats were used for this experiment. Rats were treated with either 0.9% saline or cocaine (20 mg/kg, i.p.) once daily for 5 consecutive days and were immediately returned to their home cage. On days 6-9, the animals were drug free and remained in the home cage. On day 10, the effect of Sal A on the expression of cocaine

sensitization and stereotypy were measured. On the test day, animals were habituated in the activity chamber for 30 min. Rats that were pre-treated with either saline or cocaine for 5 consecutive days were injected with either vehicle (75% DMSO) or Sal A (0.3 mg/kg, i.p.) followed, 5 min later, by cocaine (20 mg/kg, i.p.). Locomotor activity and stereotypic counts were measured for 90 min (30 min pre-treatment + 60 min post-treatment). The dose of cocaine was selected based on previous reports which showed that cocaine administration (20 mg/kg, i.p.), once daily for 5 consecutive days produced motor sensitization in rats (Heidbreder *et al.*, 1995; 1996).

Conditioned taste aversion (CTA)

Conditioned taste aversion was performed on 13 drug naïve rats, following modified methods of Schenk *et al.*, 1987 and Fenu *et al.*, 2005. Rats were initially placed on a 23 h water deprivation schedule. The amount of water consumed (ml) during the remaining hour was measured on a daily basis. This process was repeated until the variation in water consumption was ≤2 ml for three consecutive days. The following day, rats were provided with a novel tasting 0.1% saccharin solution. During saccharin consumption sessions, rats were presented with the saccharin solution for 40 min and the total amount of saccharin consumed was measured. Animals were matched on consumption of saccharin and put into treatment groups. These animals were injected with either vehicle (75% DMSO) or Sal A (0.3 mg/kg, i.p.) and returned back to their home cage. On the test day, which was 48 h after the saccharin consumption session, rats were again presented with the novel saccharin solution for 40 min. The amounts of saccharin consumed (ml) on the pairing day and test day by Sal A-treated rats were compared with the vehicle treated animals.

Forced Swim Test (FST)

This test was conducted on 12 drug naïve rats following the method described by Porsolt *et al.* (1979) with modifications made by Detke *et al.* (1995) and Carlezon *et al.* (2006). On day 1, drug naive rats were habituated to swimming in a FST chamber (44 cm tall, 20 cm internal diameter) for 15 min. The following day, rats were injected with either vehicle (75% DMSO) or Sal A (0.3 mg/kg) and 5 min later, the FST was carried out for a period of 5 min. Forced swimming behavior was recorded by a camera connected to an adjacent computer and later scored in 5 sec intervals as climbing, swimming or immobile. The videos were analyzed by an observer who was blind to the experimental procedures.

Drugs

Cocaine HCl (Merck Pharmaceuticals, Palmerston North, New Zealand) was dissolved in 0.9% saline. Sal A isolated by Dr. Thomas E. Prisinzano (University of Kansas, Kansas, USA) was suspended in 75% DMSO. All solutions were administered i.p. with the final volume made up to 1 ml/kg. All drug weights refer to salt.

Statistical analysis

Data are expressed as mean + SEM for locomotion tests, CTA and FST experiments. Statistical analysis for cocaine-induced locomotion, behavioral sensitization and stereotypy experiments (for total ambulatory counts) were performed using separate one-way ANOVAs followed by Tukey post hoc tests. For time course analysis, two-way ANOVAs (treatment × time) with repeated measures on time were performed, followed by Bonferroni post hoc tests. Statistical analysis for stereotypic counts were also performed on total pooled counts obtained in the 20 min period before and 20 min following cocaine treatment by the Cocaine/Veh/Coc- and Cocaine/Sal A/Coc-treated groups, using the Mann-Whitney test. Data from the spontaneous locomotion test were analyzed using Student t-tests. For CTA, one-way ANOVA followed by Tukey post hoc test was used. Each behavior in the FST

(climbing, swimming and immobile) was analyzed using the Mann-Whitney test. Statistical significance was set at $p<0.05$.

Results

Effect of Sal A on spontaneous locomotion and cocaine induced hyperactivity

No significant difference was observed in spontaneous locomotion between Sal A (0.3 mg/kg) and vehicle (75% DMSO) pre-treated rats (Fig. 1). A single injection of vehicle or Sal A pre-treatment had no significant effect on total locomotor activity produced following a saline (1 ml/kg) injection (Fig. 2a). A significant increase in total locomotor activity was observed in rats pre-treated with vehicle or Sal A followed by an injection of cocaine (20 mg/kg) [F (3, 22) = 21.52, $P < 0.001$]. Additional post hoc analysis showed that there was no significant difference in cocaine produced hyperactivity (total activity) between the Sal A and vehicle pre-treated groups (Fig. 2a). However, time-course analysis showed a significant increase in locomotor activity in Sal A-treated rats vs. vehicle-treated controls at 5, 10 and 15 min following cocaine injection ($P < 0.05$) [F (51, 396) = 5.05, $P < 0.001$; Fig. 2b]. This indicates that acute Sal A (0.3 mg/kg) increased locomotor activity during the first 15 min following cocaine (20 mg/kg) injection without modulating locomotor activity by itself. Also, post-hoc analysis indicated no significant difference in the locomotion observed during the initial 30 min habituation period (Fig. 2b).

Effect of Sal A on expression of behavioral sensitization

Animals exposed to vehicle followed by an injection of cocaine on the test day (day 10), produced a significant increase in total locomotion when compared to animals that received saline on days 1-5, indicating the expression of cocaine sensitization [F (3,23) = 3.07, $P < 0.05$; Fig. 3a]. Post-hoc tests revealed no significant difference in the total activity of animals exposed to Sal A (0.3 mg/kg) on test day when they received either saline or cocaine from day 1-5 ($P > 0.05$) (Fig. 3a). However, further time-course analysis showed a significant reduction in locomotion in Sal A-treated rats vs. vehicle-treated groups at 5, 10, 15 and 20 min following cocaine injection ($P < 0.05$) [F (51,441) = 4.0, $P < 0.001$; Fig. 3b]. This indicates that acute exposure to Sal A significantly attenuates the expression of cocaine sensitization. Post-hoc analysis showed no significant difference in the locomotion observed during the initial 30 min habituation period for rats that received either saline or cocaine on days 1-5 (Fig. 3b).

Effect of Sal A on cocaine-induced stereotypic counts

Cocaine-induced stereotypy was analyzed by measuring the number of repetitive beam breaks collected during the expression of sensitization experiment. No significant difference in the total number of stereotypic counts produced by cocaine was observed between the Sal A-pretreated and vehicle-pretreated controls [F (3,23) = 0.36, NS; Fig. 4a]. A further time-course analysis showed a significant interaction effect on cocaine produced stereotypy [F (51,414) = 1.54, $P < 0.02$; Fig. 4b] although post-hoc tests revealed no significant difference in stereotypic counts between Sal A-pre-treated and vehicle-pre-treated cocaine-sensitized animals (Fig. 4b). A trend towards an increase in the stereotypic counts was noted in Sal A treated animals in the 20 min period following cocaine injection (Fig. 4b). Further analysis on pooled stereotypic counts 20 min pre- and 20 min post-cocaine injection between Sal A-treated and vehicle-treated sensitized animals also showed no significant difference (Fig. 4c).

The effect of Sal A on conditioned taste aversion

Statistical analysis indicated no significant difference in the amount of saccharin consumed by vehicle and Sal A pre-treated animals [F (3,22) = 2.7, *NS*; Fig. 5]. However, a non-significant trend towards an increase in the amount of saccharin consumed by vehicle- and Sal A-treated groups on the pairing day vs. test day was noted (Fig. 5). Post-hoc tests showed no significant difference in the amount of saccharin consumed (ml) on test day in rats exposed to Sal A vs. vehicle-treated groups (Fig. 5). Thus, a single injection of Sal A (0.3 mg/kg) paired with a novel tasting saccharin solution did not produce conditioned taste aversion in rats.

Effect of Sal A on forced swim test

The effect of Sal A (0, 0.3 mg/kg) on climbing, swimming and immobility behaviors was measured for 5 min on the test day (Fig. 6). A significant reduction in climbing ($p<0.05$) and swimming ($p<0.01$) time, and a significant increase in time spent immobile ($p<0.01$) were observed for Sal A vs. vehicle control rats.

Discussion

An acute treatment of Sal A at 0.3 mg/kg has previously been shown to attenuate cocaine seeking (Morani *et al.*, 2009). Here we show that Sal A (0.3 mg/kg) also modulates cocaine-induced locomotor activity in rats. Sal A suppressed the expression of cocaine sensitization without affecting stereotypic counts, or causing taste aversion. However Sal A induced depressive-like behaviors in the FST. No change in spontaneous open field activity was observed. In contrast to its attenuating effect on behavioral sensitization, Sal A was shown to increase locomotor activity produced by acute cocaine exposure.

Previous studies have shown that conditioning stimuli can play an important role in the expression of motor sensitized responses in laboratory animals (Post *et al.*, 1981; Beninger and Herz, 1986). In the present study, rats that received cocaine or saline injections for 5 successive days were returned back to their home cage after drug/vehicle exposures, thereby limiting conditioned effects. Therefore, the difference in ambulation on the test day (day 10) was specifically due to the pharmacological effect of the drug on the pre-treatment days (day 1-5) (Fig. 3).

The effects of Sal A on cocaine sensitization might reflect effects on DA neurotransmission. Both the VTA and NAc have been implicated in the initiation and development of cocaine sensitization (Kalivas and Duffy, 1993a; Heidbreder *et al.*, 1996; Shippenberg *et al.*, 1996; Kalivas *et al.*, 1998; Steketee, 2005) and Sal A modulates DA levels in the dorsal (Zhang *et al.*, 2005; Gherke *et al.*, 2008) and ventral striatum (Carlezon *et al.*, 2006). The role of this mechanism in the attenuation of cocaine sensitization by Sal A is currently being investigated. Sedation and motor in-coordination are two of the commonly documented adverse effects associated with KOPr activation (Mello and Negus, 2000; Walsh *et al.*, 2001; Wadenberg 2003). Our results show that Sal A did not suppress open field activity in drug naive rats (Fig. 1), thus suggesting non-sedative effects. This finding also implies that the suppression of cocaine behavioral sensitization by Sal A was not due to non-selective effects (Fig. 3), as we have previously suggested (Morani *et al.*, 2009).

Sal A (0.3 mg/kg) potentiates cocaine (20 mg/kg)-induced hyperactivity in drug-naive rats (Fig. 2). However, at high doses, Sal A (2 mg/kg) has been shown to attenuate hyperactivity produced by a low dose of cocaine (10.0 mg/kg) (Chartoff et al., 2008). These effects may be due to prior cocaine exposures, as Sal A (0.3 mg/kg) attenuated behavioral sensitization (current study) and drug seeking in animals with previous cocaine exposures. It is possible that the attenuation of cocaine seeking seen with high doses of Sal A may reflect sedative

effects, whereas, low doses of Sal A are likely to affect the drug-seeking response directly. This idea is supported by other results that also showed that a high dose of Sal A (2.0 mg/kg) increased the intracranial self-stimulation (ICSS) threshold, suppressed sucrose reinforcement and decreased phasic DA release in NAc (Ebner *et al.*, 2010), but a lower dose had no effect on any of the measures (Ebner *et al.*, 2010). Similarly, high doses (1.0, 3.2 mg/kg) produced conditioned place aversion in mice (Zhang *et al.*, 2005).

The decrease in cocaine-induced behavioural sensitization produced by Sal A may be due to several factors. It could reflect either a decrease in the ability of cocaine to produce horizontal activity or an increase in the ability of cocaine to produce the competing behaviour of stereotypy that follows high-dose psychostimulant administration (Ushijima *et al.*, 1995; Post *et al.*, 1987). Because the dose-effect curve for activity is in the shape of an inverted U, with higher doses producing more intense stereotypy, the present data cannot distinguish between these two possibilities. Detailed studies on cocaine stereotypy and the role of Sal A in modulating this behavior are therefore required to clarify this point. The data from other paradigms, however, are consistent with the idea that Sal A decreases the response to cocaine (Chartoff et al., 2008; Morani et al., 2009).

A single exposure to Sal A does not produce taste aversion when paired with a novel tasting saccharin solution (Fig. 5). On close observation, acute Sal A exposure induced a non-significant trend towards taste preference. This trend may be due to the low dose of Sal A (0.3 mg/kg) tested in this study. Low doses of Sal A have previously been shown to produce preference to conditioned behaviors in both zebrafish and rats (Braida et al., 2007; 2008).

Previous reports have shown that Sal A produces both anti-depressant (Braida *et al.*, 2007; 2008) and pro-depressive effects in rats assessed by the FST paradigm (Carlezon *et al.*, 2006). In the current study, acute exposure to Sal A (0.3 mg/kg) produced pro-depressive behaviour (Fig. 6). Because locomotor activity was not altered with this dose of Sal A (Fig. 1), the effects are probably not attributable to motoric disruption. These results are consistent with the findings of Carlezon *et al.*, (2006). In contrast to these findings, Braida *et al.*, (2009) showed that acute Sal A (up to 1 mg/kg) exposure produced anti-depressant effects in rats using the FST. The observable dissimilarities may be attributed to the differences in the route of administration for Sal A (i.p. current study, Carlezon *et al.*, 2006 vs. s.c. Braida *et al.*, 2009) and the duration of Sal A pre-treatment (5 min, current study vs. 20 min Braida *et al.*, 2009). Differences in the vehicle used to suspend Sal A are also noted, which may change the availability of Sal A (75% DMSO, current study, Carlezon *et al.*, 2006 vs. 1:1:8, Ethanol: Tween 80: water, Braida *et al.*, 2009).

Animals pre-treated with Sal A showed significant reductions in time spent in both climbing and swimming, as has previously been reported (Carlezon *et al.*, 2006). A decrease in climbing time has been attributed to effects at the norepinephrine transporter (NET), whereas modulation of serotonin transporters (SERT) are implicated in the reduction in swimming time (Detke *et al.*, 1995). There are no reports on whether KOPrs mediate the modulation of NET. However, previous reports have shown that KOPr activation decreases serotonin levels in brain regions implicated in depressive behaviours (Tao and Auerbach, 2005; Yilmaz *et al.*, 2006). KOPr activation has also been shown to increase cyclic AMP response element binding protein (CREB) phosphorylation in the NAc, which is an important marker for depression (Carlezon *et al.*, 1998; Nestler and Carlezon, 2006). This effect is antagonised by SERT, NET or KOPr inhibition (Mague *et al.*, 2003; Chartoff *et al.*, 2009). Thus, these effects might reflect KOPr agonist-induced effects on serotonin and/or norepinephrine systems.

The KOPr system has been implicated in the modulation of the hedonic effects produced by cocaine. It has been suggested previously that activation of KOPr may prevent the development and progression of cocaine addiction during the initial stages of the addiction cycle (Shippenberg et al., 2001; 2007; Chefer et al., 2005; Mysels and Sullivan, 2009; Bruijnzeel, 2009). However, adverse effects such as sedation, aversion and depression have prevented their clinical development (Walsh et al., 2001). Results from this study establish the role of Sal A in antagonizing cocaine produced behaviors in the rat with fewer adverse effects. These findings support the development of novel neoclerodane diterpenes as anti-cocaine agents. However, further work is necessary to identify the mechanism by which Sal A produces its anti-cocaine- and depressive- effects.

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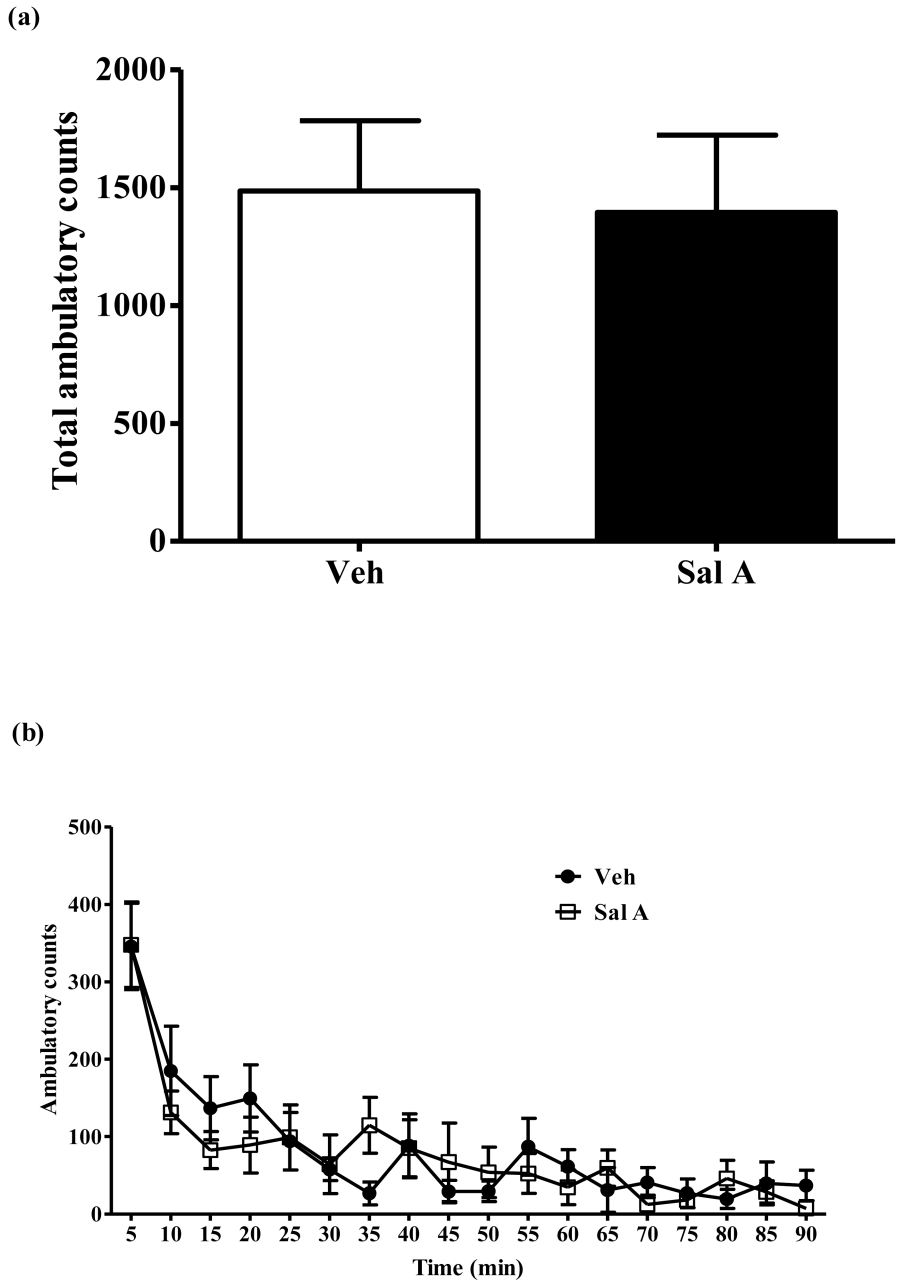

Fig. 1.

Effect of salvinorin A (Sal A) on spontaneous locomotion. Animals were habituated to the locomotion boxes for 30 min followed by vehicle or Sal A treatment and activity counts were measured for 60 min. Symbols indicate (a) Mean total activity (+ SEM) and (b) mean (± SEM) locomotor activity measured at 5 min intervals for 90 min. Student t-test. n= 7 per group.

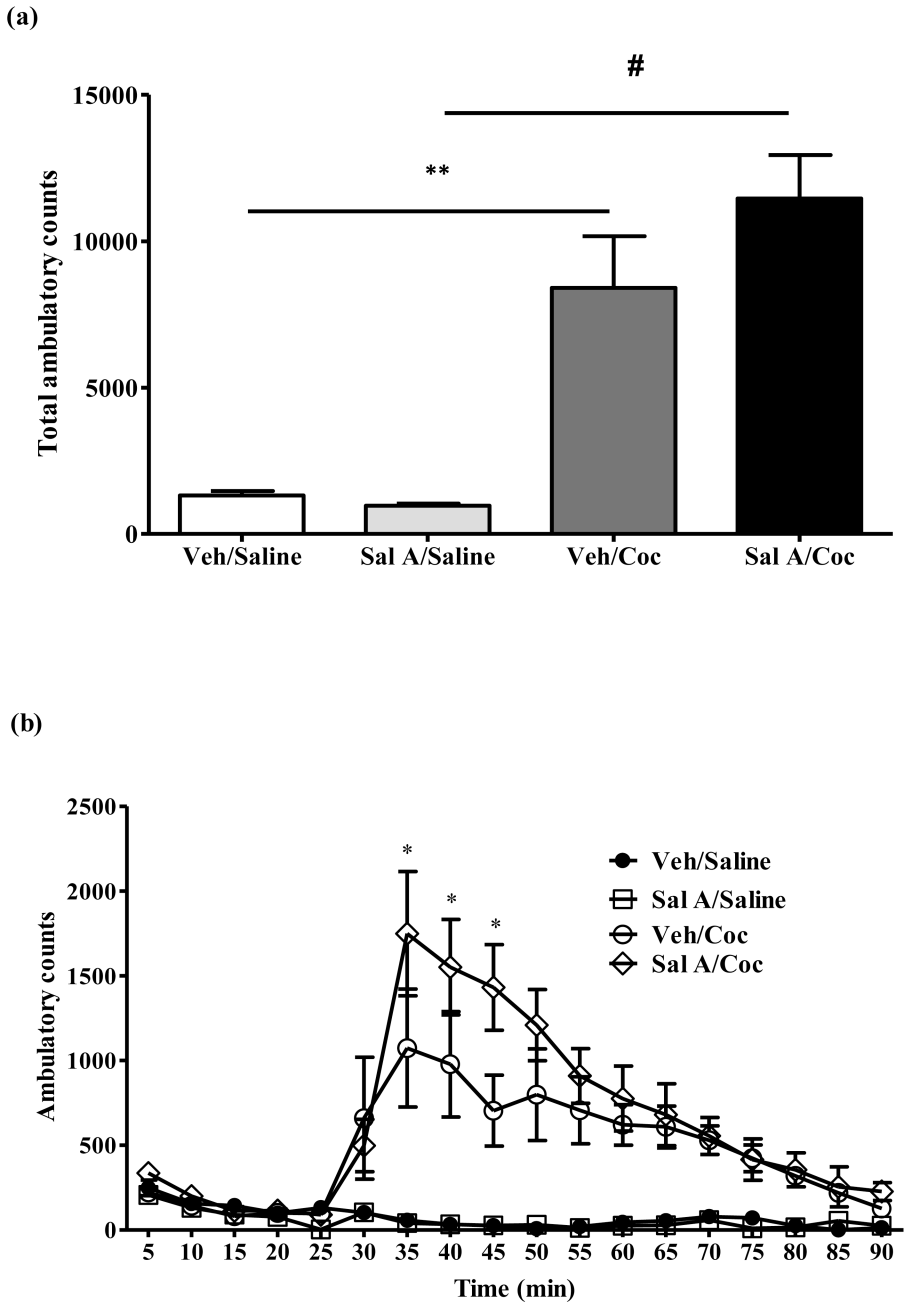

Fig. 2.

Effect of salvinorin A (Sal A) on cocaine induced hyperactivity. Drug naive rats were initially habituated in the locomotion boxes for 30 min. Animals were later injected with either vehicle (Veh, 75% DMSO) or Sal A (0.3 mg/kg) followed by saline (1 ml/kg) or cocaine (Coc, 20 mg/kg) and locomotor activity was monitored for 60 min. (a) 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. (b) Time-course measurement of mean (± SEM) locomotor activity at 5 min intervals. *$p<0.05$, vs. Veh/Coc treated group: repeated-measures two-way ANOVA followed by Bonferroni post hoc test. n= 6-7 per group.

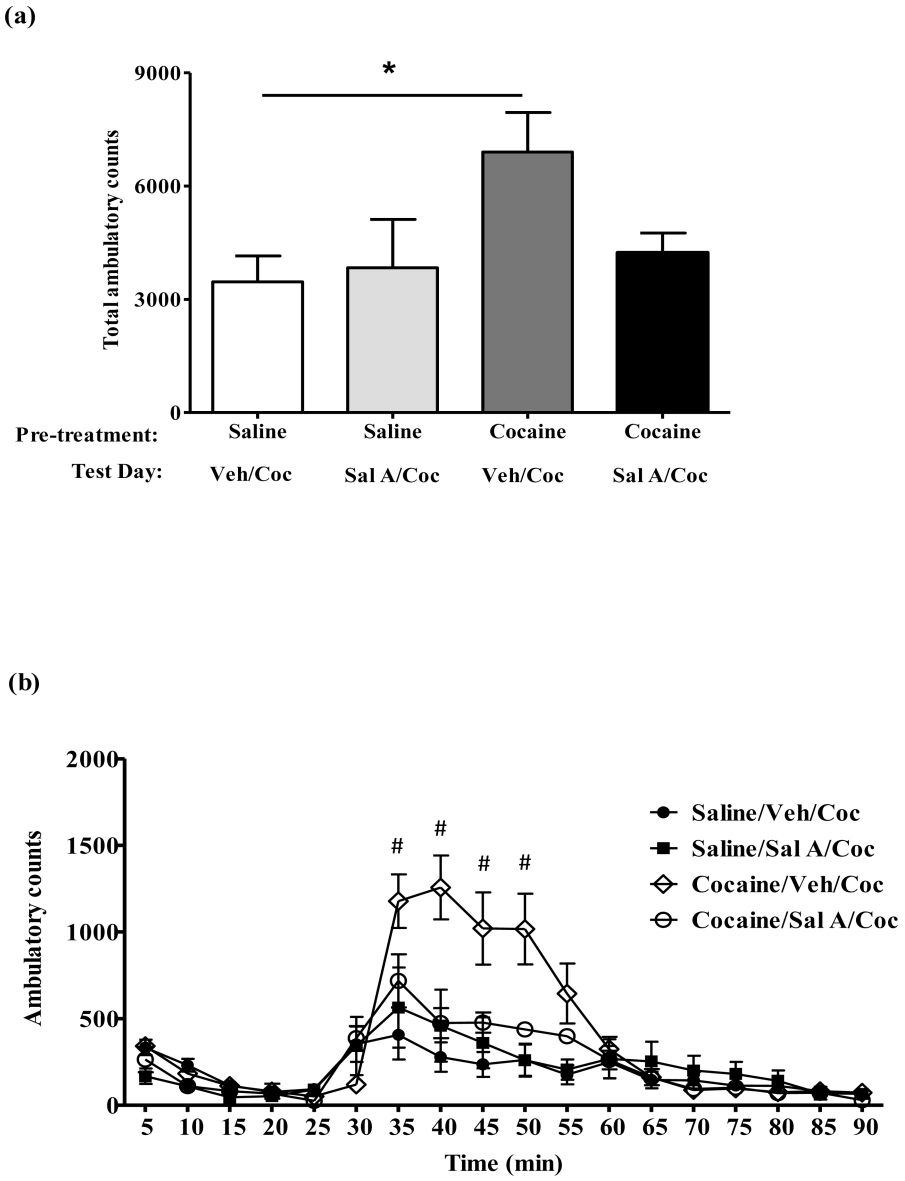

Fig. 3.

Effect of salvinorin A (Sal A) on the 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. (a) Data expressed as mean total activity (+SEM). *$p<0.05$, data compared with Saline/Veh/Coc treated group: one-way ANOVA followed by Tukey test. (b) Time-course measurement of mean (± SEM) of locomotion activity at 5 min intervals. *$p<0.05$, vs. Cocaine/Sal A/Coc treated group: repeated-measures two-way ANOVA followed by Bonferroni post hoc test. n= 6-8 per group.

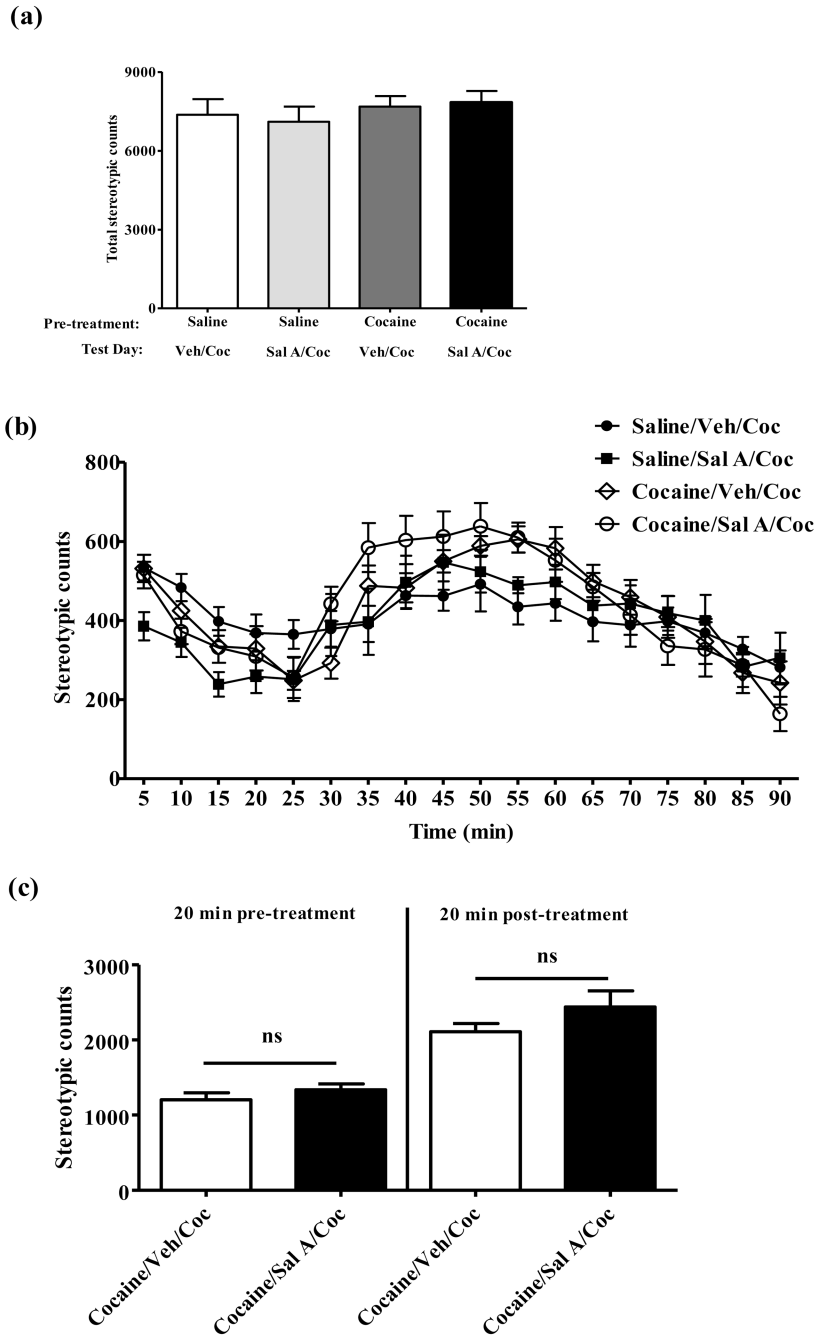

Fig. 4.

Effect of Sal A on cocaine induced stereotypy. Data obtained from rats subjected to the expression of cocaine behavioral sensitization experiments were also analyzed for cocaine-produced stereotypic counts. (a) Bars indicate mean total stereotypic counts (+SEM).: one-way ANOVA followed by Tukey test. (b) Time-course measurements of mean (±SEM) stereotypic counts at 5 min intervals for 90 min: repeated-measures two-way ANOVA followed by Bonferroni post hoc test. (c) Bars indicate the pooled average (+SEM) of stereotypic counts at 20 min pre-and 20 min post-cocaine treatment: ns, non-significant, data compared with Cocaine/Veh/Coc vs. Cocaine/Sal A/Coc treated group; Mann-Whitney test. n= 6-8 per group.

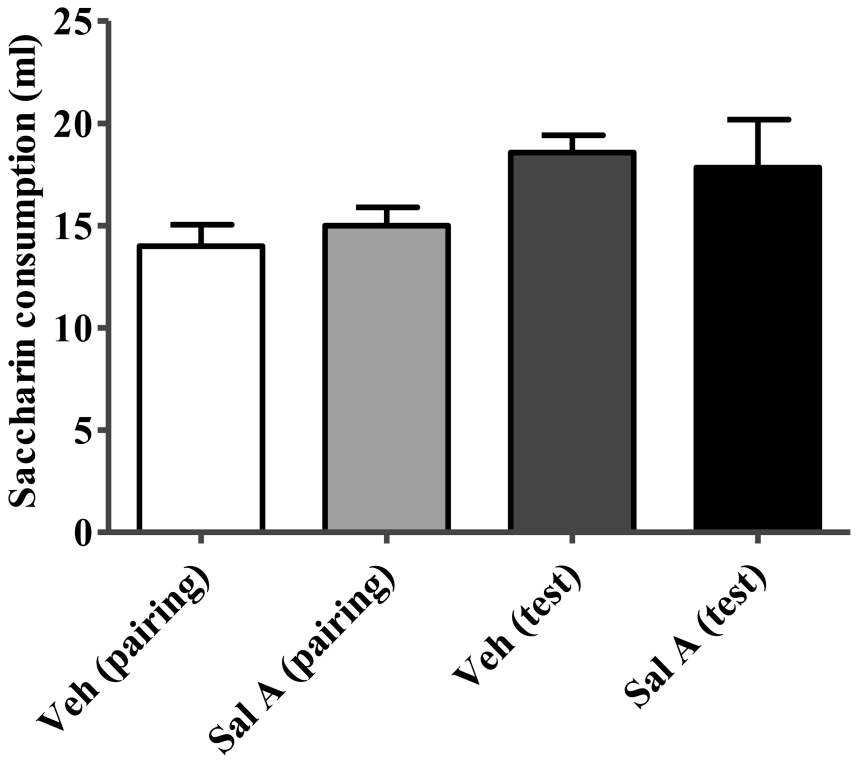

Fig. 5.

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 pairing and test day: one-way ANOVA followed by Tukey test. n = 6-7 per group.

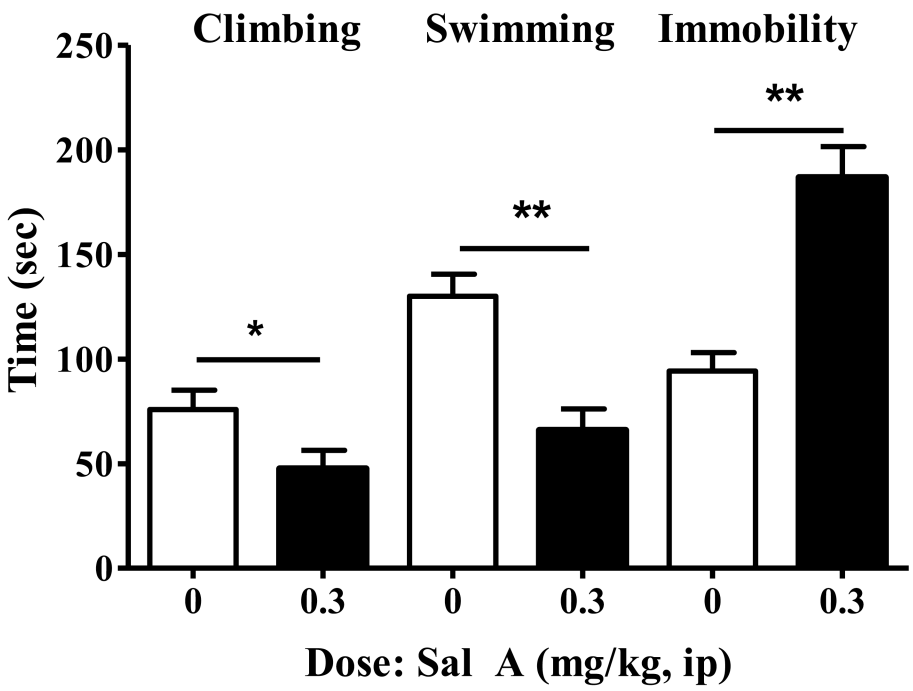

Fig. 6.

Effect of a single injection of salvinorin A (Sal A) on the forced swim test (FST) in drug naive rats (n=6). On the test day, animals were injected with Sal A and 5 min later were subjected to the 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 per group.