Psychopharmacology (2015) 232:91–100
DOI 10.1007/s00213-014-3641-z

ORIGINAL INVESTIGATION

Behavioural and neurochemical assessment of salvinorin A abuse potential in the rat

Veronica Serra · Liana Fattore · Maria Scherma · Roberto Collu · Maria Sabrina Spano · Walter Fratta · Paola Fadda

Received: 16 December 2013 / Accepted: 22 May 2014 / Published online: 13 June 2014

Abstract
Rationale Salvinorin A is a recreational drug derived from *Salvia divinorum*, a sage species long used as an entheogen. While salvinorin A has potent hallucinogenic properties, its abuse potential has not been assessed consistently in controlled behavioural and neurochemical studies in rodents.
Objective This study aimed to assess salvinorin A abuse potential by measuring its capacity to establish and maintain self-administration behaviour and to modify dopamine (DA) levels in the nucleus accumbens (NAcc) of rats.
Results Male Lister Hooded (LH) and Sprague-Dawley (SD) rats were allowed to self-administer salvinorin A (0.5 or 1.0 μg/kg/infusion) intravenously 2 h/day for 20 days under a continuous schedule of reinforcement and lever pressing as *operandum*. LH rats discriminated between the active and inactive levers but did not reach the acquisition criterion for stable self-administration (≥12 active responses vs ≤5 inactive responses for at least 5 consecutive days). SD rats discriminated between the two levers at the lower dose only but, like LH rats, never acquired stable self-administration behaviour. Systemic salvinorin A increased extracellular DA in the NAcc shell of both LH (at ≥40 μg/kg) and SD rats (at ≥5 μg/kg), but injection into the ventral tegmental area (VTA) induced no significant change in NAcc DA concentration in LH rats and only brief elevations in SD rats.
Conclusions Salvinorin A differs from other commonly abused compounds since although it affects accumbal dopamine transmission, yet it is unable, at least at the tested doses, to sustain stable intravenous self-administration behaviour.

Keywords Salvinorin A · Drug abuse · Addiction · Self-administration · Dopamine · Microdialysis

V. Serra · L. Fattore · M. Scherma · R. Collu · M. S. Spano · W. Fratta · P. Fadda
Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Cittadella Universitaria di Monserrato, 09042 Monserrato, CA, Italy

L. Fattore · W. Fratta · P. Fadda
Center of Excellence "Neurobiology of Addiction", University of Cagliari, Cittadella Universitaria di Monserrato, 09042 Monserrato, CA, Italy

L. Fattore
CNR Institute of Neuroscience-Cagliari, National Research Council-Italy, University of Cagliari, Cittadella Universitaria di Monserrato, 09042 Monserrato, CA, Italy

W. Fratta · P. Fadda (✉)
National Institute of Neuroscience (INN), University of Cagliari, Cittadella Universitaria di Monserrato, 09042 Monserrato (CA), Italy
e-mail: pfadda@unica.it

V. Serra
Institute of Translational Pharmacology, UOS of Cagliari, National Research Council, Scientific and Technological Park of Sardinia - Polaris, Pula, Italy

Introduction

Salvia divinorum, the only psychotropic species among the approximately 1,000 species of sage (genus *Salvia*), has been long used in religious and healing ceremonies by the Mazatec Indians to induce a state of "divine inebriation" (Ott 1995). Neuropsychological effects of *S. divinorum* include alterations of mood, behaviour, and cognition. It possesses potent hallucinogenic effects in humans according to descriptive studies and self-reports (Johnson et al. 2011; Siebert 1994). The active ingredient salvinorin A is one of the most potent naturally occurring hallucinogens known, with effects comparable to lysergic acid diethylamide (LSD), but unlike most "classic" plant hallucinogens (e.g., psilocybin), it does not bind to the 5-HT2A receptor (Roth et al. 2002).

Some of the subjective effects of salvinorin A are distinct from other hallucinogens or dissociative compounds (Addy 2012), possibly because it possesses a peculiar pharmacological profile, which includes potent and selective binding to the κ-opioid receptor (Roth et al. 2002). Although structurally distinct from classical κ-opioid agonists (John et al. 2006; McCurdy et al. 2006a, b), salvinorin A shares with them same pharmacological effects, including depression (Carlezon et al. 2006), antinociception (John et al. 2006), sedation (Zhang et al. 2005), discriminative stimulus effects (Baker et al. 2009), and conditioned place aversion (Zhang et al. 2005). Alternatively, more recent studies have reported antidepressant-like effects (Braida et al. 2009; Harden et al. 2012) but also learning and memory impairments (Braida et al. 2011). Salvinorin A was also found to allosterically modulate human μ-opioid receptors expressed in Chinese hamster ovary cells (Rothman et al. 2007), stimulate the dopamine type 2 (D2) receptor in rat striatal tissue (Seeman et al. 2009), and modulate noradrenaline (NA), serotonin (5-HT), and dopamine (DA) exocytosis from rat synaptosomes by acting at presynaptic opioid receptors (Grilli et al. 2009).

Recent surveys reported recreational use of *S. divinorum* by 4.4 % to 6.4 % of students at a southwestern American university (Lange et al. 2008) and that heavy marijuana smoking was the strongest predictor of *S. divinorum* use (Khey et al. 2008). Recreational use in industrialised countries (UNODC 2013) has finally captured the attention of the scientific community (Wu et al. 2011). Since it supposedly has low abuse potential, *S. divinorum* is not under international control and few studies have characterised the behavioural effects and abuse potential of *S. divinorum* or salvinorin A in either animals or humans.

Earlier studies revealed that high doses of salvinorin A decreased DA levels in selected brain areas of rats and mice and induced conditioned place aversion in mice (Gehrke et al. 2008; Zhang et al. 2005). Salvinorin A was also shown to decrease evoked phasic DA release in the nucleus accumbens (NAcc) without affecting DA reuptake (Ebner et al. 2010). At very low doses, salvinorin A induced accelerated swimming behaviour and conditioned place preference in the zebra fish, intracerebroventricular self-administration in rats, while at high doses, it induced a "trance-like state" (Braida et al. 2007, 2008). These data suggest that salvinorin A, at low doses similar to those consumed by *S. divinorum* smokers, is likely to induce positive reinforcing effects.

To further characterise the potential reinforcing effects of salvinorin A, we investigated whether this compound shares with other drugs of abuse the capacity to sustain intravenous self-administration (IVSA) in rats and increase DA (or DA metabolites) concentration in the NAcc shell after systemic or intra-ventral tegmental area (VTA) injection. Strain-dependent differences in the response to different drugs of abuse have been reported, including self-administration behaviour (Deiana et al. 2007; Shoaib et al. 1997), which may help to reveal molecular mechanisms leading to abuse. Therefore, we evaluated the potential positive reinforcing effects of salvinorin A in two rat strains, Lister Hooded (LH) and Sprague-Dawley (SD).

Materials and methods

Animals

Male Lister Hooded and Sprague-Dawley rats (Harlan Nossan, Italy) weighing 250–270 g at the beginning of the study were housed six per cage under standard environmental conditions (temperature 21±1 °C, humidity 60 %) and given 1 week of acclimation and handling before surgery.

For IVSA experiments, rats were housed under a reversed 12-h light/dark cycle (lights on at 7:00 p.m.). Rats were maintained on 18 g/*die* of standard chow with water available *ad libitum* for the entire duration of the study. In line with our previous studies assessing animal responses to drugs with low abuse potential, such as cannabinoids (Fattore et al. 2001; Martellotta et al. 1998), behavioural experiments were conducted during the dark phase of the cycle when animals typically display better acquisition and performance on operant tasks (Gritton et al. 2012).

Rats undergoing microdialysis experiments were housed under a normal 12-h light/dark cycle (lights on at 7:00 a.m.) and given *ad libitum* access to food and water. In line with previous studies assessing mesolimbic DA levels after salvinorin A administration (Ebner et al. 2010; Gehrke et al. 2008; Zhang et al. 2005), neurochemical measurements were conducted during the light phase of the cycle.

All experiments were approved by the University of Cagliari Committee on Animal Use and Care and performed in accordance with the E.C. regulations for animal use in research (86/609/EEC).

Drugs

Salvinorin A (Tocris, Italy) was dissolved in a 1:1:8 solution containing ethanol (Carlo Erba, Italy), Tween 80 (Merck, Italy), and 0.9 % sterile saline. For IVSA experiments, drug doses of 0.5 and 1.0 μg/kg/inf were used in a fixed volume of 100 μl. To ensure sterility, drug solutions were filtered through 0.22-μm syringe filters prior to use.

For microdialysis experiments, salvinorin A was administered either subcutaneously (sc) at 5, 10, and 40 μg/kg or directly into the VTA at 0.125, 0.25, 0.5, and 1 μg/2 μl. All antibiotics and anaesthetics were purchased as sterile solutions from local distributors. Doses of salvinorin A selected for this study were within the range inducing reward (Braida et al.

2008) but below those inducing depressant effects on behaviour and neurochemistry in rats (Carlezon et al. 2006).

Intravenous self-administration

Under deep anaesthesia by intraperitoneal (ip) administration of Equithesin (0.97 g pentobarbital, 2.1 g Mg sulphate, 4.25 g chloral hydrate, 42.8 ml propylene glycol, 11.5 ml ethanol 90 %, 5 ml/kg), animals were surgically implanted with a permanent intravenous catheter (CamCaths, Cambridge, UK) in the right jugular vein as described (Fattore et al. 2001). Following catheter placement, animals were individually housed and allowed 6 or 7 days of recovery under antibiotic treatment (Baytril, Bayer, 0.1 ml, sc). Catheters were flushed daily with heparinized (1 %) sterile solution to ensure patency.

Intravenous self-administration experiments were conducted in operant chambers (29.5×32.5×23.5 cm), each encased in a sound- and light-attenuating box provided with a ventilation fan (Med Associates, St Albans, VT, USA). Each chamber was equipped with two 4-cm wide retractable levers positioned 12 cm apart, 8 cm from the grid floor, and extending 1.5 cm into the box. The box also contained a white stimulus light (cue light) located between the two levers and a red light (home light) located on the opposite wall. Intravenous infusions of salvinorin A were controlled by a software-operated pump (Med Associates, USA) mounted outside the box and connected via an extra length of silastic tubing to a single-channel swivel mounted on a counterbalanced arm to allow animals to move freely in the chamber. A length of plastic tubing enclosed in a metal spring connected the swivel to the catheter fitting on the animal's back. The assignment of the active (drug-paired) and inactive levers was counterbalanced within groups but remained the same for each individual rat throughout the study.

A single press of the active lever resulted in a 5-s contingent infusion of 100 μl drug solution and concurrent presentation of the cue light. A 15-s timeout period was then introduced, during which further presses on the active lever had no consequence. Depression of the inactive lever had no programmed consequences but was always recorded. Each chamber was equipped with infrared locomotion sensors placed on each wall at regular intervals 3.5 cm from the grid floor. The number of beam breaks was recorded and used as a measure of general horizontal activity. Assessment of the self-administration schedule and all other data collection procedures were controlled by Med Associates PC software.

All animals were allowed to self-administer salvinorin A (0.5 or 1 μg/kg) in daily 2-h sessions under a continuous (FR-1) schedule of reinforcement and lever pressing as *operandum*. The IVSA sessions were conducted during the dark phase of the cycle (0900 to 1200 hours), 6 days/week. The IVSA response was considered stable when animals displayed accurate discrimination between the active and inactive lever as defined by a number of active lever presses >10 active lever presses with≤20 % daily variation over 5 consecutive days.

In vivo microdialysis

Animals were anaesthetised with Equithesin (5 ml/kg, ip), placed in a stereotaxic head frame (David Kopf Instruments, Tujunga, CA, USA) and secured using blunt ear bars. The skull was exposed, and a small hole drilled on the right side. A concentric microdialysis probe with 2 mm dialyzing surface length (AN 69AF; Hospal-Dasco, Bologna, Italy; cutoff 40,000 Da, in vitro recovery about 30 %) was inserted vertically into the shell of the NAcc at AP, +2; ML, ±1; and DV, −8 for SD rats and AP, +1.6; ML, ±1.1; and DV, −7.9 for LH rats (coordinates relative to bregma in mm). For LH rats, the mouth bar was set to −3.3 flat skull) as previously described (Fadda et al. 2003, 2006). The probe was fixed to the skull using acrylic dental cement. For local VTA injections, the coordinates (mm relative to bregma) were AP, −4.7; ML, ±0.7; and DV, −8.2 in SD rats and AP, −5; ML, ±1.8; and DV, −7.6 in LH rats. After surgery, animals were single housed with *ad libitum* access to food and water.

The apparatus for high-pressure liquid chromatography (HPLC) was equipped with an isocratic pump (ESA model 580), injector (Rheodyne 7125), reverse-phase column (LC 18 DB Supelco 4.6×150 mm), and ESA Coulochem II detector with the first electrode set at +400 mV and the second at −180 mV. The mobile phase used to analyse metabolites consisted of CH_3COONa 50 mM, EDTA 0.07 mM, OSA 0.35 mM, and MeOH 12 %, adjusted to pH 4.21 using CHCOOH. Flow rate was 1 ml/min. Under these conditions, assay sensitivity for DA was 2 fmol for each sample. At 24 h post-surgery, the dialysis probe was perfused at a constant flow rate of 2.5 μl/min with artificial cerebrospinal fluid (aCSF) (NaCl 147 mM, $CaCl_2$ $2H_2O$ 1.5 mM, KCl 4 mM, pH 6.0–6.5) using a CMA/100 microinjection pump (Carnegie, Medicine, Sweden). Dialysate samples (50 μl) were collected every 20 min and directly injected into the HPLC system to evaluate the levels of DA and DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (HIAA).

Once a stable basal level of DA was reached (mean ± SEM of three consecutive samples not differing by more than 10 % from each other), animals were administered salvinorin A and samples were collected every 20 min for 180 min. The location of the probe was determined histologically at the end of each experiment on coronal brain section (50 μm) stained with cresyl violet. All experiments were performed between 8:00 a.m. and 4:00 p.m. Only results acquired from rats with correctly positioned dialysis probes were included in the statistical analysis.

Statistical analysis

Self-administration experiment Lever-pressing activity during the 20-day IVSA training was compared between the active and inactive levers by two-way ANOVA for repeated measures. If a statistically significant difference was detected, the Bonferroni *post hoc* test was used for individual comparisons. Student's *t* test was used to compare mean daily motor activity (number of beam breaks) between strains and active vs. inactive lever presses within each daily session. A $p<0.05$ was considered significant.

Microdialysis experiment All samples collected post-treatment were expressed as percent change relative to the final three basal values (100 %) and then averaged for all rats within each treatment group (expressed as mean ± SEM%). Statistical significance of drug treatment effects was determined using one-way analysis of variance (ANOVA) for single curve analysis (one dose) or two-way ANOVA for comparison among the three doses and vehicle. Tukey's test was used for *post hoc* comparison, with the statistical significance set at $p<0.05$.

Results

Intravenous self-administration

To examine the abuse potential of salvinorin A, rats from both the LH and SD strains were tested for acquisition of self-administration behaviour by measuring the mean (±S.E.M.) number of active and inactive lever presses over 20 days (Fig. 1). At the lower dose of salvinorin A tested (0.5 μg/kg/inf), LH rats did not display clear acquisition of drug self-administration over time. During the first 10 days of training, active lever response frequency was highly variable, although it remained significantly higher than inactive level response frequency on 6 of the first 9 days ($p<0.02$; Fig. 1a). From day 11 to day 16, LH rats showed more consistent operant behaviour but there was no difference between active and inactive lever response rate. During the last days of training, the number of active lever presses gradually diminished to reach a nadir by day 20 (Fig. 1a). More prolonged training did not rescue the active lever response, which remained consistently≤4/session (data not shown). Two-way ANOVA for repeated measures detected a main effect of lever choice (active vs. inactive) ($F_{1,360}=21.47$; $p<0.0001$), but no significant effect of training day ($F_{19,360}=0.57$; $p=0.9237$) or lever choice×day interaction ($F_{19,360}=0.77$; $p=0.9940$). Similarly, when infusion of the higher dose of salvinorin A (1.0 μg/kg/inf) was triggered by the active lever (Fig. 1b), LH animals failed to develop and sustain IVSA behaviour. LH rats did self-administer the high dose frequently during the first 4 days of training (mean of 11–13 responses/session), but inactive lever pressing frequency was also high (5–9 responses/session) such that the difference was significant on only one of these days (day 4). Active lever pressing remained relatively stable and not significantly higher than inactive response frequency over the subsequent 13 sessions (except for 1 day) before declining from day 18 onward. Again, prolonging the training period did not rescue the active lever response (data not shown). Animals did not display a clear discrimination between the active and the inactive lever (means, 9.53±2.7 vs. 6.34±1.9). Two-way ANOVA for repeated measures revealed a main effect of lever choice ($F_{1,520}=18.11$; $p<0.0001$) but no effect of day ($F_{19,520}=0.94$; $p=0.5274$) or a lever choice×day interaction ($F_{19,520}=0.53$; $p=0.9499$). Despite the main effect of lever choice, LH rats did not reach the criterion for stable salvinorin A self-administration (≥10 active responses with a variation <20 % for 5 consecutive days).

At the lower salvinorin A dose of 0.5 μg/kg/inf, SD animals performed significantly more active than inactive lever presses on most of the first 9 training days (4.1±1.5 vs. 1.4±1.9; Fig.1c), and two-way ANOVA revealed a main effect of lever choice ($F_{1,360}=47.50$; $p<0.0001$) and day ($F_{19,360}=2.04$; $p<0.01$) but no lever choice×day interaction ($F_{19,360}=0.37$; $p=0.9940$). As in the case of LH rats, SD rats did not attain the threshold criterion for stable self-administration behaviour. Increasing the dose to 1.0 μg/kg/inf (Fig.1d) actually reduced lever discrimination (mean active lever response/session, 3.59±1.1 for the active and 2.72±1.3 for the inactive lever). Two-way ANOVA revealed no effect of lever ($F_{1,320}=2.95$; $p=0.0870$), day ($F_{19,320}=0.73$; $p=0.7851$), or lever×day interaction ($F_{19,320}=1.07$; $p=0.3822$). Thus, SD rats did not acquire self-administration behaviour at either dose.

Motor activity

Mean daily spontaneous motor activity in LH and SD rats during the 20-day self-administration training period was then examined (Fig. 2). Lister Hooded rats displayed significantly greater motor activity when given access to 1.0 μg/kg/inf compared to the lower dose ($p<0.0014$, *t* test), consistent with the greater mean number of active and inactive lever responses compared to the lower-dose condition. In contrast to the LH strain, SD rats showed similar motor activity during IVSA training at both doses ($p<0.985$, *t* test). No strain-dependent differences were found in the level of motor activity between LH (Fig. 2a) and SD (Fig. 2b) rats, indicating that drug-induced locomotor effects cannot account for the strain differences in response rates observed during salvinorin A self-administration. Notably, within each experimental group, motor activity of animals was rather constant throughout all training sessions, for the very initial to the last

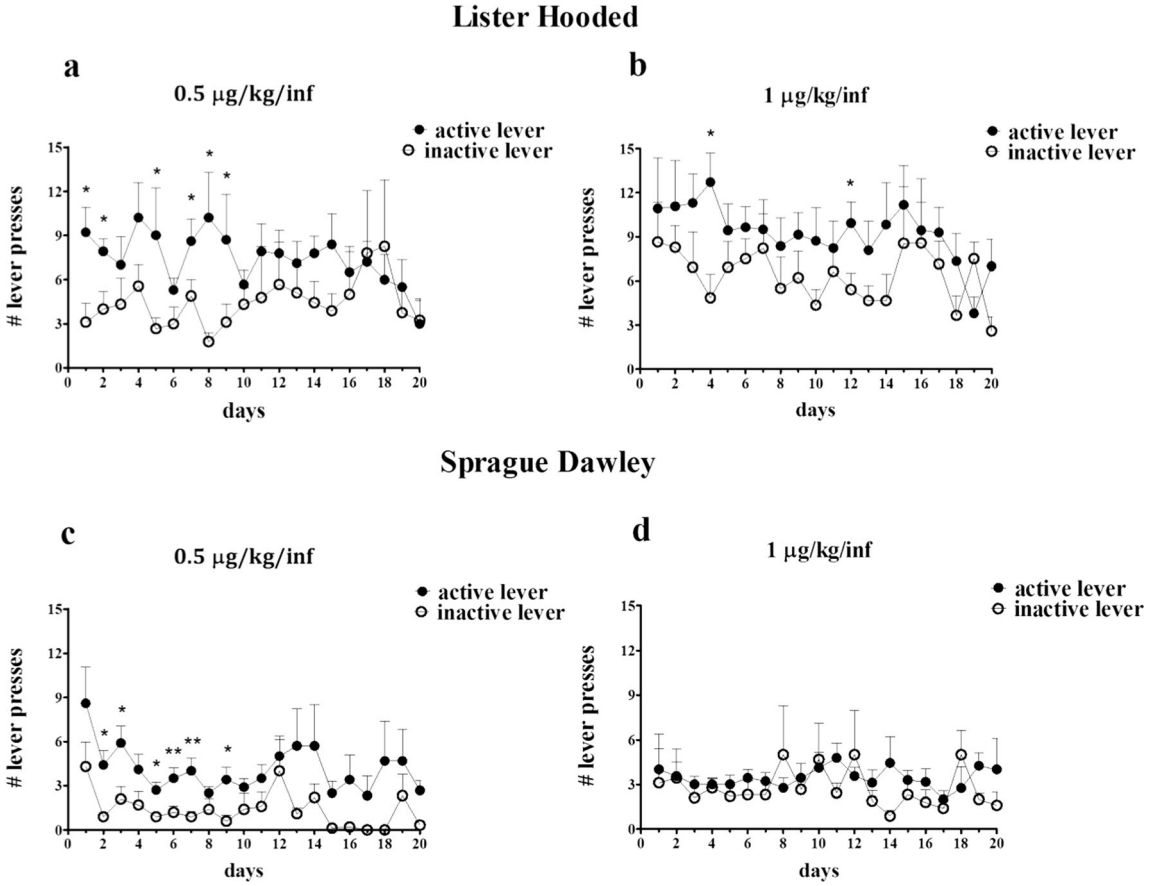

Fig. 1 Intravenous self-administration of salvinorin A 0.5 (**a** and **c**) and 1 μg/kg/inf (**b** and **d**) in LH rats (**a**, **b**) and SD rats (**c**, **d**). Values are expressed as mean ± SEM of active and inactive lever presses during each daily 2 h session. Animals per group, $n=10$ for LH and SD rats in 0.5 μg/kg/inf salvinorin A dose groups (**a** and **c**), $n=14$ for LH rats in the 1 μg/kg/inf dose group (**b**), $n=9$ for SD rats in the 1 μg/kg/inf dose group (**d**). *$p<0.02$ and **$p<0.005$, Student's *t* test

training sessions, independently from the dose of salvinorin A self-administered and the specific strain considered (data not shown).

Effect of subcutaneous administration of salvinorin A on the release of DA and DA metabolites in the NAcc

Self-administration is strongly associated with the capacity of a given drug to increase DA within the NAcc. We measured extracellular levels of DA (Fig. 3a), DOPAC (3b), HVA (3c) and 5-HIIA (3d) in the shell of the NAcc of LH rats over 3 hours following subcutaneous administration of salvinorin A (5, 10, and 40 μg/kg). Two-way ANOVA revealed a main effect of dose ($F_{3,160}=8.77$, $p=0.0001$) and a dose×time interaction ($F_{27,160}=1.66$, $p=0.0291$) but no main effect of time ($F_{9,160}=1.01$, $p=0.4$). Only at the highest dose did salvinorin A induce a small but statistically significant increase in extracellular DA level relative to vehicle-treated rats (Fig. 3a), which occurred about 120 mins after drug injection (one-way ANOVA, $F_{9,27}=7.53$, $p=0.0001$). The effect on DA was not accompanied by any change in DOPAC (b), HVA (c) or 5-HIIA levels (d). Two-way ANOVA revealed no effects of dose, time or dose×time on the NAcc concentration of any of these metabolites.

In SD rats (Fig. 4), all salvinorin A doses significantly increased extracellular DA levels in the NAcc compared to vehicle (Fig. 4a) but had no effects on metabolite concentrations (Fig. 4b–d). In contrast to the delayed effect of salvinorin A on NAcc DA in LH rats, 10 μg/kg caused an almost immediate and significant increase in extracellular DA level in the SD rat NAcc that persisted for at least 180 min. At the lower and higher doses of 5 and 40 μg/kg, salvinorin A determined a rapid increase in DA levels that lasted for 100 and 60 min, respectively, after drug injection (Fig. 4a). Two-way ANOVA revealed a significant main effect of dose ($F_{3,230}=21.08$, $p<0.0001$) but no time ($F_{9,230}=1.90$, $p>0.05$) or dose×time interaction ($F_{27,230}=0.91$, $p>0.05$). One-way ANOVA on individual dose-response curves revealed significant DA increases with time ($F_{9,72}=4.572$, $p=0.0002$; $F_{9,69}=10.43$, $p<0.0001$; $F_{9,36}=13.54$, $p<0.0001$).

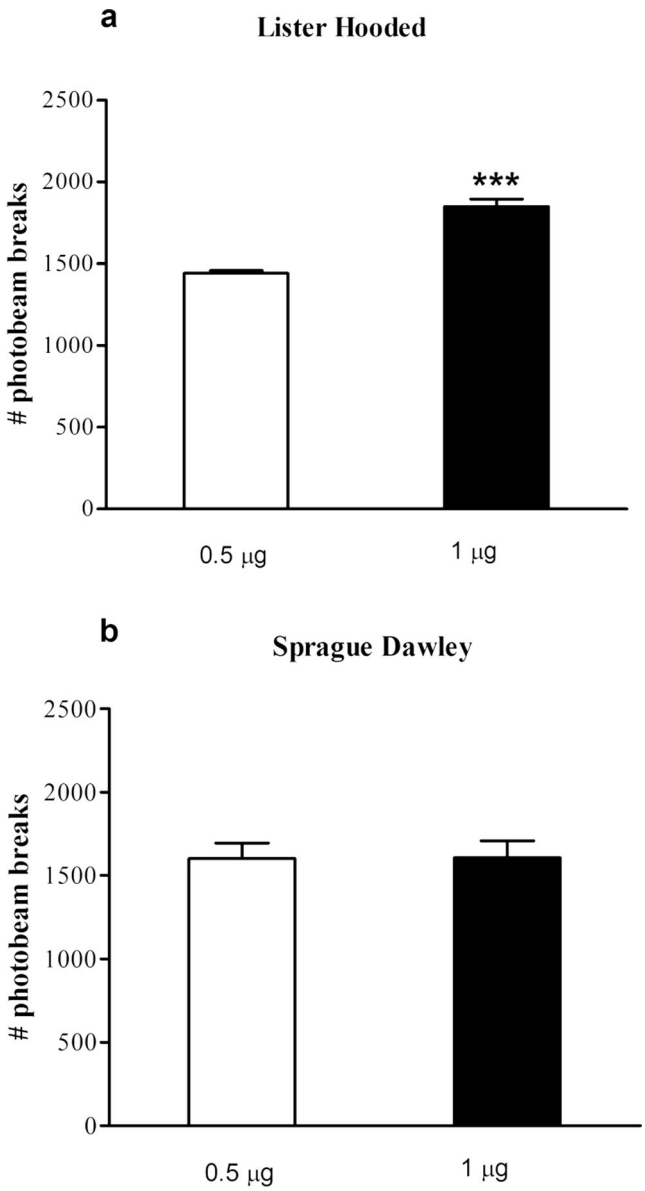

Fig. 2 Motor activity by LH (**a**) and SD (**b**) rats during IVSA sessions of salvinorin A at 0.5 μg/kg/inf (*white bars*) and 1.0 μg/kg/inf (*black bars*). Motor activity is expressed as mean ± SEM total photobeam breaks over the 20 days of IVSA training in each strain. All groups include the same number of animals used for the IVSA experiments. ***$p<0.0001$, Student's *t* test

Effect of intra-VTA administration of salvinorin A on the release of DA in the NAcc

We then tested whether intra-VTA administration of salvinorin A (0.125, 0.25, 0.5, or 1.0 μg/2 μl) altered extracellular DA in the NAcc (Fig. 5). In LH rats, two-way ANOVA revealed no main effect of dose, time, or dose×time on DA concentration compared to vehicle (Fig. 5a). Similarly, one-way ANOVA on each dose-response curve revealed no significant effect. In SD rats, intra-VTA administration of salvinorin A led to an oscillation in DA levels within the NAcc at all doses tested (Fig. 5b), but these changes did not reach statistical significance compared to vehicle by two-way ANOVA with the exception of two time points (80 and 120 min) at 0.5 μg/2 μl ($p<0.05$, one-way ANOVA).

Discussion

Addiction is characterised by the transition from occasional to compulsive consumption of determinate compounds, generally associated with a loss of control in the amount of the drug administered. It is now well established that addictive substances produce reinforcing effects by acting on the mesolimbic dopaminergic system, a major regulator of emotion, motivation and mood (Fibiger 1978; Wise 1978). The dopaminergic hypothesis of addiction is based on evidence that the five major classes of addictive compounds (psychostimulants, opioids, cannabinoids, ethanol and nicotine) act by altering dopamine transmission in the mesolimbic pathway (Wise 2004a, b; Wise and Bozarth 1987). Salvinorin A is classified as a hallucinogen, a class of compounds for which a clear demonstration of addictive properties is still lacking. However, although salvinorin A induces subjective effects similar to classic hallucinogens, including "mystical" entheogenic effects (Johnson et al. 2011), its pharmacological profile differs from that of other classic hallucinogens like LSD and mescaline. For example, Salvinorin A binds with high affinity to the κ-opioid receptor (Roth et al. 2002), stimulates the D2 receptor (Seeman et al. 2009) and does not generalise with hallucinogenic compounds like LSD, ketamine or psilocybin (Butelman et al. 2010; Killinger et al. 2010). Recently, the use of salvinorin A has increased in industrialised countries, leading to greater interest in assessing its addictive potential.

Our data do not support strong addictive potential, at least at the doses tested, in LH and SD rats. Under our experimental conditions, neither strain demonstrated stable IVSA behaviour within 20 days, although both showed modest discrimination between the two active and inactive levers. In contrast to present data, salvinorin A was shown to support stable intake in the intracranial self-administration paradigm (Braida et al. 2008). In addition to drug delivery route, other differences in experimental conditions, such as dose, injection volume, or duration of infusion, could impact the propensity for stable self-administration behaviour. It is also possible that the specific rat strains studied, LH and SD, are not able to acquire stable responding for salvinorin A. Indeed, cannabinoid IVSA behaviour can be established only in certain rat strains (Deiana et al. 2007) and within a limited dose range (Fattore et al. 2001). In support of this, the rewarding effects of salvinorin A demonstrated by Braida and collaborators by intracranial self-administration paradigm were observed in the Wistar rat

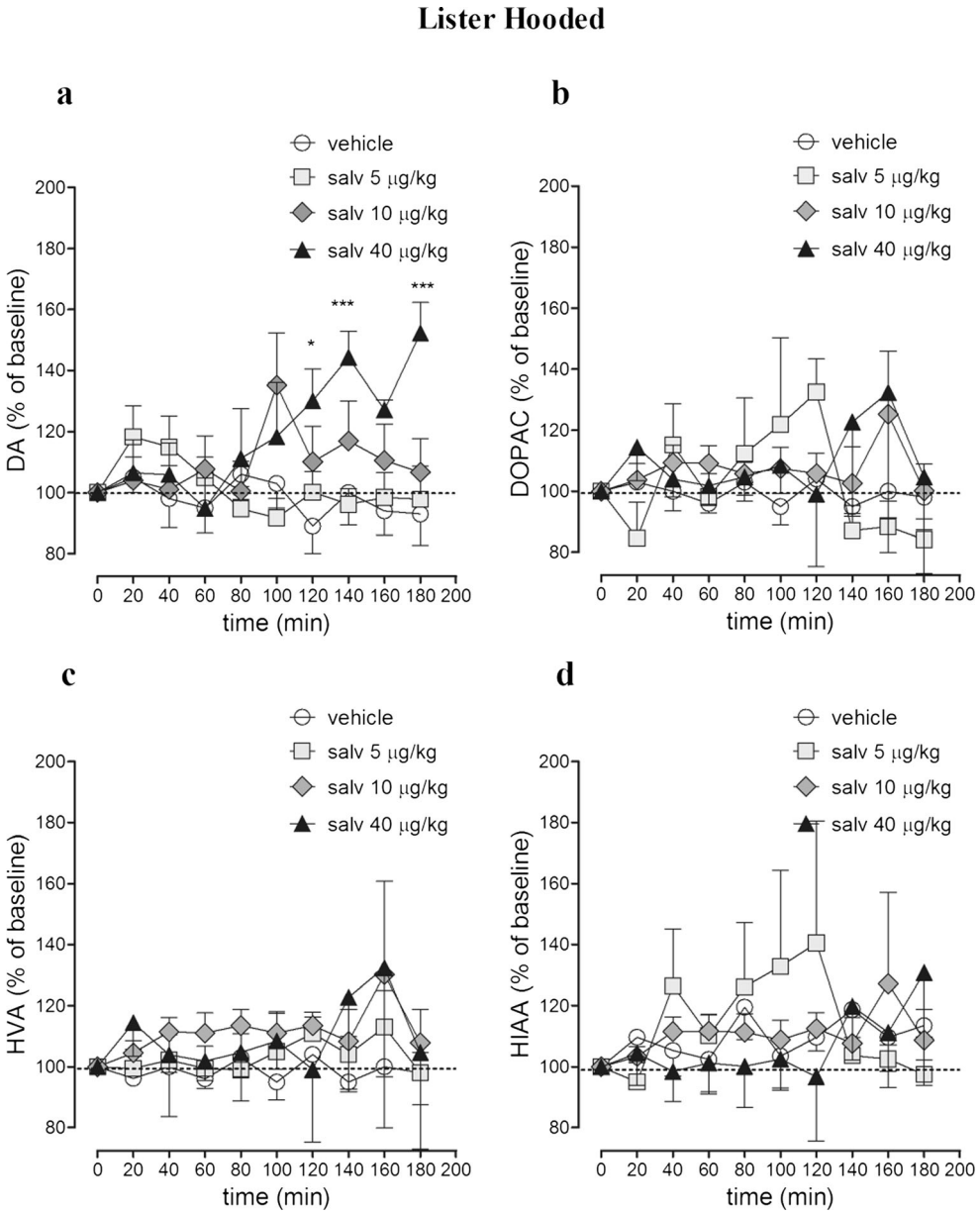

Fig. 3 Effect of salvinorin A (5, 10, 40 μg/kg sc) on the extracellular concentration of DA (**a**), DOPAC (**b**), HVA (**c**) and HIAA (**d**) in the shell of the NAcc of LH rats. Values are the mean ± SEM percent variation of basal values per group (n=5 for vehicle and 5 μg/kg salvinorin A; n=6 for 10 μg/kg salvinorin A; n=4 for 40 μg/kg salvinorin A). *p<0.05 and ***p<0.0001

strain, thus supporting the hypothesis that salvinorin A induces its rewarding effects at different extent depending on animal strains. The effects of salvinorin A on additional rat strains or on female rats warrant further investigation, as such studies may identify genetic factors regulating abuse potential. Notably, human subjects commonly exhibit sporadic rather than regular use of salvinorin A, a habit that has been justified by the lack of immediate euphoric effects (Ranganathan et al. 2012) as well as by the occurrence of cognitive deficits (Braida et al. 2011) and acute strong dissociative and memory impairments (MacLean et al. 2013).

In 2005, Zhang and colleagues demonstrated a reduction in DA levels in the rat caudate–putamen and NAcc after intraperitoneal administration of salvinorin A. These data were confirmed 1 year later using a different protocol, which also showed no fluctuations in 5-HT levels (Carlezon et al. 2006). Salvinorin A was also shown to decrease phasic DA release in the Nacc core and shell in association with a decrease in motivation (Ebner et al. 2010). Conversely, using very low doses of the drug (micrograms rather than milligrammes), it has been demonstrated that salvinorin A increased DA levels in the NAcc of rats (Braida et al. 2008) although to a lesser extent than observed with known addictive compounds (Di Chiara and Imperato 1988a, b). Microdialysis experiments performed in this study showed that salvinorin A, with the only exception of the subcutaneous dose of 40 μg/kg, was not able to modify DA extracellular levels in the Nacc shell of LH rats when administered systemically or locally in the VTA. On the contrary, all doses tested administered systemically, but not intra-VTA, were able to increase DA release in the Nacc

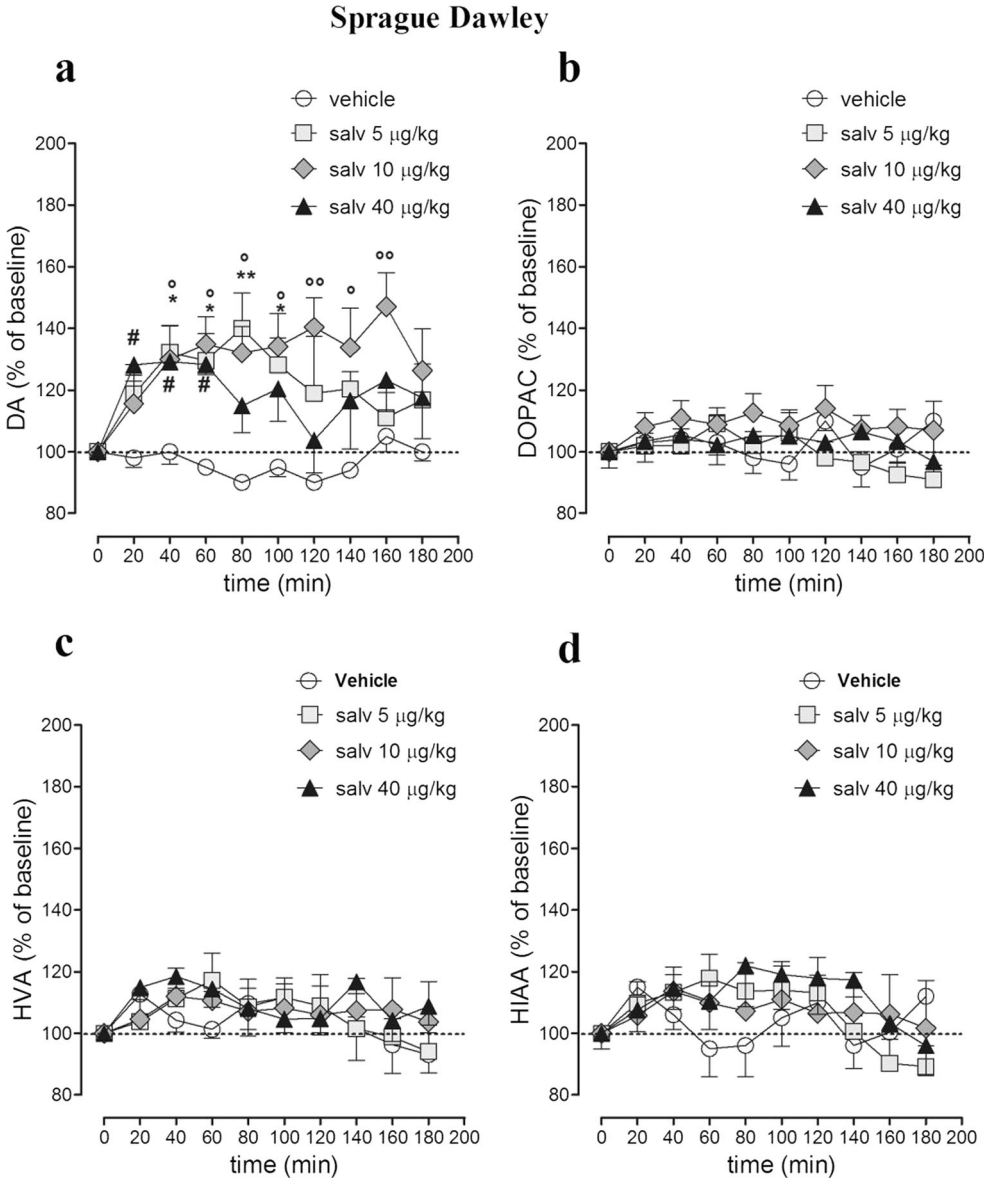

Fig. 4 Effect of salvinorin A administration (5, 10, 40 μg/kg sc) on the extracellular concentration of DA (**a**), DOPAC (**b**), HVA (**c**) and HIAA (**d**) in the shell of the NAcc of SD rats. Values are the mean ± SEM percent variation of basal values per group (n=6 for vehicle (Veh), n=9 for 5 μg/kg salvinorin A, n=7 for 10 μg/kg salvinorin A, n=5 for 40 μg/kg salvinorin A). *p<0.05 and **p<0.001 5 μg/kg salvinorin A vs. Veh, °p<0.05 and °°p<0.001 10 μg/kg salvinorin A vs. Veh, $^{\#}p$<0.05 40 μg/kg salvinorin A vs. Veh

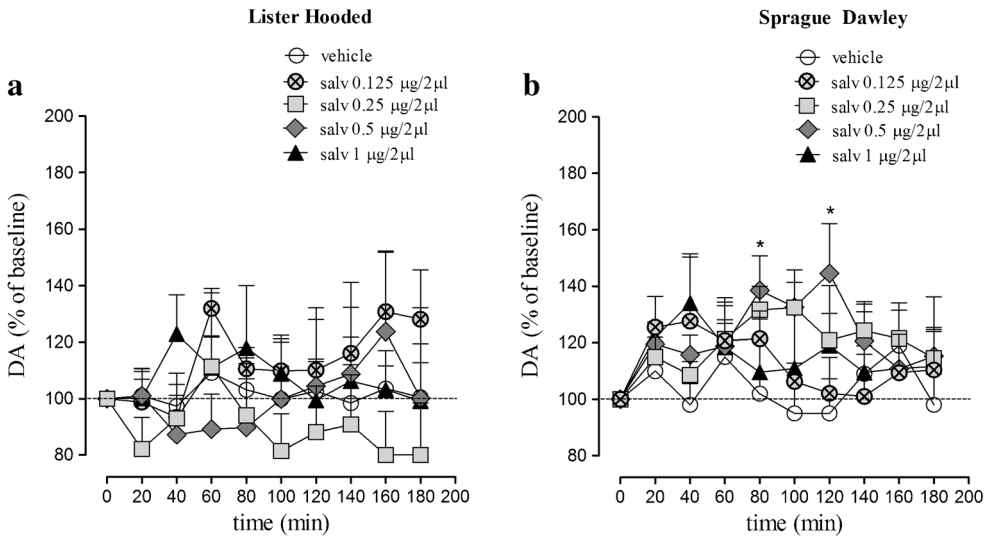

Fig. 5 Effect of intra-VTA administration of salvinorin A (0.125, 0.25, 0.5 and 1 μg/2 μl) on the extracellular concentration of DA in the NAcc shell of LH (**a**) and SD (**b**) rats. Values are the mean ± SEM percent variation of basal values per group (LH, n=4 for vehicle, 0.125 and 0.5 μg/2 μl salvinorin A; n=7 for 0.25 μg/2 μl salvinorin A; n=6 for 1 μg/2 μl salvinorin A; SD, n=4 for vehicle and 0.125 μg/2 μl; n=6 for 0.25 μg/2 μl salvinorin A; n=10 for 0.5 μg/2 μl salvinorin A; n=8 for 1 μg/2 μl salvinorin A). *p<0.05 for 0.5 μg/2 μl salvinorin A vs. vehicle

shell of SD rats although not in a dose-dependent manner. The lack of effect on DA transmission after intra-VTA administration in both strains may be due to the fact that salvinorin A acts mainly as a κ-opioid agonist. As well documented, dopaminergic neurotransmission in the mesolimbic pathway is regulated by opioids, and administration of kappa receptor agonists generally decrease DA extracellular levels in NAcc (Di Chiara and Imperato 1988b; Spanagel et al. 1990). In our study, we tested very low doses of salvinorin A, doses that may be not capable of determining any modification in terms of DA release. However, the increase of DA release observed here and in previous studies (Braida et al. 2008) following systemic administration can be accounted to the fact that salvinorin A may exert its actions through activation of one or more as yet undiscovered molecular target (Zawilska and Wojcieszak 2013).

In conclusion, we showed that salvinorin A is not able to establish self-administration behaviour in two different strains of rats, at least within the doses tested, but is able to slightly modify DA levels in the rat shell NAcc in both strains of rats. We cannot exclude, however, the possibility that under different experimental conditions (e.g. extended access to the drug, non-contingent primings before the sessions, etc) and using different doses of the drug, animals might display a stable volitional intake of salvinorin A. Nonetheless, the pharmacological effects of salvinorin A warrant further investigation in light of the possible induction of psychosis in susceptible subjects or frequent users (Meyer and Writer 2012; Przekop and Lee 2009) due to activation of D2 receptors (Seeman et al. 2009), a property shared by street drugs of abuse (Seeman et al. 2006). Moreover, use of *S. divinorum* among recent or active drug users is associated with a high prevalence of substance use disorders (Wu et al. 2011), emphasising the need to study health risks arising from drugs interactions.

Acknowledgments Funding for this study was provided by the Italian Ministry of University and Scientific Research (Progetti di Ricerca a rilevante Interesse Nazionale, PRIN 2006); the authors are grateful to Dr. Barbara Tuveri and Marta Tuveri for animal care and technical assistance. This manuscript is dedicated to the memory of Dr. Maria Sabrina Spano.

References

Addy PH (2012) Acute and post-acute behavioural and psychological effects of salvinorin A in humans. Psychopharmacology 220:195–204

Baker LE, Panos JJ, Killinger BA, Peet MM, Bell LM, Haliw LA, Walker SL (2009) Comparison of the discriminative stimulus effects of salvinorin A and its derivatives to U69,593 and U50,488 in rats. Psychopharmacology 203:203–211

Braida D, Limonta V, Pegorini S, Zani A, Guerini-Rocco C, Gori E, Sala M (2007) Hallucinatory and rewarding effect of salvinorin A in zebrafish: kappa-opioid and CB1-cannabinoid receptor involvement. Psychopharmacology 190:441–448

Braida D, Limonta V, Capurro V, Fadda P, Rubino T, Mascia P, Zani A, Gori E, Fratta W, Parolaro D, Sala M (2008) Involvement of kappa-opioid and endocannabinoid system on Salvinorin A-induced reward. Biol Psychiatry 63:286–292

Braida D, Capurro V, Zani A, Rubino T, Vigano D, Parolaro D, Sala M (2009) Potential anxiolytic- and antidepressant-like effects of salvinorin A, the main active ingredient of Salvia divinorum, in rodents. Br J Pharmacol 157:844–853

Braida D, Donzelli A, Martucci R, Capurro V, Sala M (2011) Learning and memory impairment induced by salvinorin A, the principal ingredient of Salvia divinorum, in wistar rats. Int J Toxicol 30:650–661

Butelman ER, Rus S, Prisinzano TE, Kreek MJ (2010) The discriminative effects of the kappa-opioid hallucinogen salvinorin A in nonhuman primates: dissociation from classic hallucinogen effects. Psychopharmacology 210:253–262

Carlezon WA Jr, Beguin C, DiNieri JA, Baumann MH, Richards MR, Todtenkopf MS, Rothman RB, Ma Z, Lee DY, Cohen BM (2006) Depressive-like effects of the kappa-opioid receptor agonist salvinorin A on behaviour and neurochemistry in rats. J Pharmacol Exp Ther 316:440–447

Deiana S, Fattore L, Spano MS, Cossu G, Porcu E, Fadda P, Fratta W (2007) Strain and schedule-dependent differences in the acquisition, maintenance and extinction of intravenous cannabinoid self-administration in rats. Neuropharmacology 52:646–654

Di Chiara G, Imperato A (1988a) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85:5274–5278

Di Chiara G, Imperato A (1988b) Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J Pharmacol Exp Ther 244:1067–1080

Ebner SR, Roitman MF, Potter DN, Rachlin AB, Chartoff EH (2010) Depressive-like effects of the kappa opioid receptor agonist salvinorin A are associated with decreased phasic dopamine release in the nucleus accumbens. Psychopharmacology 210:241–252

Fadda P, Scherma M, Fresu A, Collu M, Fratta W (2003) Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. Synapse 50:1–6

Fadda P, Scherma M, Spano MS, Salis P, Melis V, Fattore L, Fratta W (2006) Cannabinoid self-administration increases dopamine release in the nucleus accumbens. Neuroreport 17:1629–1632

Fattore L, Cossu G, Martellotta CM, Fratta W (2001) Intravenous self-administration of the cannabinoid CB1 receptor agonist WIN 55, 212-2 in rats. Psychopharmacology 156:410–416

Fibiger HC (1978) Drugs and reinforcement mechanisms: a critical review of the catecholamine theory. Annu Rev Pharmacol Toxicol 18:37–56

Gehrke BJ, Chefer VI, Shippenberg TS (2008) Effects of acute and repeated administration of salvinorin A on dopamine function in the rat dorsal striatum. Psychopharmacology 197:509–517

Grilli M, Neri E, Zappettini S, Massa F, Bisio A, Romussi G, Marchi M, Pittaluga A (2009) Salvinorin A exerts opposite presynaptic controls on neurotransmitter exocytosis from mouse brain nerve terminals. Neuropharmacology 57:523–530

Gritton HJ, Kantorowski A, Sarter M, Lee TM (2012) Bidirectional interactions between circadian entrainment and cognitive performance. Learn Mem 19:126–141

Harden MT, Smith SE, Niehoff JA, McCurdy CR, Taylor GT (2012) Antidepressive effects of the κ-opioid receptor agonist salvinorin A in a rat model of anhedonia. Behav Pharmacol 23:710–715

John TF, French LG, Erlichman JS (2006) The antinociceptive effect of salvinorin A in mice. Eur J Pharmacol 545:129–133

Johnson MW, MacLean KA, Reissig CJ, Prisinzano TE, Griffiths RR (2011) Human psychopharmacology and dose-effects of salvinorin

A, a kappa opioid agonist hallucinogen present in the plant Salvia divinorum. Drug Alcohol Depend 115:150–155

Khey DN, Miller BL, Griffin OH (2008) Salvia divinorum use among a college student sample. J Drug Educ 38:297–306

Killinger BA, Peet MM, Baker LE (2010) Salvinorin A fails to substitute for the discriminative stimulus effects of LSD or ketamine in Sprague-Dawley rats. Pharmacol Biochem Behav 96:260–265

Lange JE, Reed MB, Croff JM, Clapp JD (2008) College student use of Salvia divinorum. Drug Alcohol Depend 94:263–266

MacLean KA, Johnson MW, Reissig CJ, Prisinzano TE, Griffiths RR (2013) Dose-related effects of salvinorin A in humans: dissociative, hallucinogenic, and memory effects. Psychopharmacology 226: 381–392

Martellotta MC, Cossu G, Fattore L, Gessa GL, Fratta W (1998) Self-administration of the cannabinoid receptor agonist WI 55,212-2 in drug-naive mice. Neuroscience 85:327–330

McCurdy CR, Sufka KJ, Smith GH, Warnick JE, Nieto MJ (2006) Antinociceptive profile of salvinorin A, a structurally unique kappa opioid receptor agonist. Pharmacol Biochem Behav 83:109–113

Meyer EG, Writer BW (2012) Salvia divinorum. Psychosomatics 53: 277–279

Ott J (1995) Ethnopharmacognosy and human pharmacology of Salvia divinorum and salvinorin A. Curare 18:103–129

Przekop P, Lee T (2009) Persistent psychosis associated with salvia divinorum use. Am J Psychiatry 166:832

Ranganathan M, Schnakenberg A, Skosnik PD, Cohen BM, Pittman B, Sewell RA, D'Souza DC (2012) Dose-related behavioral subjective, endocrine, and psychophysiological effects of the k opioid agonist Salvinorin A in humans. Biol Psychiatry 72:871–879

Roth BL, Baner K, Westkaemper R, Siebert D, Rice KC, Steinberg S, Ernsberger P, Rothman RB (2002) Salvinorin A: a potent naturally occurring non nitrogenous kappa opioid selective agonist. Proc Natl Acad Sci U S A 99:11934–11939

Rothman RB, Murphy DL, Xu H, Godin JA, Dersch CM, Partilla JS, Tidgewell K, Schmidt M, Prisinzano TE (2007) Salvinorin A: allosteric interactions at the mu-opioid receptor. J Pharmacol Exp Ther 320:801–810

Seeman P, Schwarz J, Chen JF, Szechtman H, Perreault M, McKnight GS, Roder JC, Quirion R, Boksa P, Srivastava LK, Yanai K, Weinshenker D, Sumiyoshi T (2006) Psychosis pathways converge via $D2^{high}$ dopamine receptors. Synapse 60:319–346

Seeman P, Guan HC, Hirbec H (2009) Dopamine $D2^{High}$ receptors stimulated by phencyclidines, lysergic acid diethylamide, salvinorin A, and modafinil. Synapse 63:698–704

Shoaib M, Schindler CW, Goldberg SR (1997) Nicotine self-administration in rats: strain and nicotine pre-exposure effects on acquisition. Psychopharmacology 129:35–43

Siebert DJ (1994) Salvia divinorum and salvinorin A: new pharmacologic findings. J Ethnopharmacol 43:53–56

Spanagel R, Herz A, Shippenberg TS (1990) The effects of opioid peptides on dopamine release in the nucleus accumbens: an in vivo microdialysis study. J Neurochem 55:1734–1740

United Nations Office on Drugs and Crime (UNODOC) World drug Report 2013

Wise RA (1978) Catecholamine theories of reward: a critical review. Brain Res 152:215–247

Wise RA (2004a) Dopamine and food reward: back to the elements. Am J Physiol Regul Integr Comp Physiol 286:R13

Wise RA (2004b) Rewards wanted: molecular mechanisms of motivation. Discov Med 4:180–186

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469–492

Wu LT, Woody GE, Yang C, Li JH, Blazer DG (2011) Recent national trends in Salvia divinorum use and substance-use disorders among recent and former Salvia divinorum users compared with nonusers. Subst Abuse Rehabil 2:53–68

Zawilska JB, Wojcieszak J (2013) Salvia divinorum: from Mazatec medicinal and hallucinogenic plant to emerging recreational drug. Hum Psychopharmacol 28:403–412

Zhang Y, Butelman ER, Schlussman SD, Ho A, Kreek MJ (2005) Effects of the plant-derived hallucinogen salvinorin A on basal dopamine levels in the caudate putamen and in a conditioned place aversion assay in mice: agonist actions at kappa opioid receptors. Psychopharmacology 179:551–558