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# Salvinorin A fails to substitute for the discriminative stimulus effects of LSD or ketamine in Sprague–Dawley rats

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ABSTRACT

*Salvia divinorum* is a small perennial shrub that has gained recent popularity among the drug-using subculture as a legal alternative to hallucinogens. Salvinorin A, the main active compound found in the *S. divinorum* plant, is an atypical hallucinogen with pharmacological selectivity at kappa opioid (KOP) receptor sites and is a unique non-nitrogenous neoclerodane diterpene which is structurally distinct from other opioid compounds. The novel structure of salvinorin A and its specific binding affinity to KOP receptors provide a unique opportunity to investigate neurochemical mechanisms of hallucination and hallucinogenic compounds. The current investigation assessed the substitution of salvinorin A in 16 male Sprague–Dawley rats trained to discriminate either the prototypical serotonergic hallucinogen, LSD (0.08 mg/kg, S.C., $n=8$) or the dissociative anesthetic and glutamatergic hallucinogen, ketamine (8.0 mg/kg, I.P., $n=8$) from vehicle under a FR 20 schedule of food-reinforced responding. Results indicated that neither LSD nor ketamine discrimination generalized to salvinorin A. These findings are consistent with the growing body of evidence that salvinorin A is pharmacologically distinct from other traditional hallucinogenic compounds.



## 1. Introduction

*Salvia divinorum* is a small perennial shrub native to Mexico that has gained recent popularity in the United States and Europe as a legal hallucinogen. The use of *S. divinorum* is not exclusive to the modern world, but has been used for centuries as a divining agent by the native tribes of Oaxaca, Mexico (Wasson, 1962). The recent increase in *S. divinorum* use is presumably due to the widespread availability of both the plant material and highly potent extracts that can be readily purchased through the internet (Drug Enforcement Administration, 2008; Prisinzano, 2005). Recreational use of this substance is rampant within the internet community of YouTube©, where one can view a multitude of videos documenting recreational use of *S. divinorum* and the resulting intoxication.

When *S. divinorum* leaves are smoked or chewed as a quid, the user often experiences a "loss of awareness," which could result in users hurting themselves or others (Gonzalez et al., 2006). In reaction to the growing abuse issue, several states within the U.S. and some European countries have banned the cultivation, use, and distribution of the plant (Siebert, 2007). Recently, the Drug Enforcement Administration has cited *S. divinorum* as a "drug of concern" (Drug Enforcement Administration, 2008) and U.S. federal regulation of *S. divinorum* is a possibility in the near future.

Although recent media attention has primarily focused on the legality and abuse liability of *S. divinorum*, the unique pharmacological profile of salvinorin A, the main active compound found in *S. divinorum*, has gained considerable interest among the scientific community. Salvinorin A was first isolated from this plant by Ortega et al. in 1982 and it remains the most potent naturally occurring hallucinogen known to mankind. Salvinorin A is a unique furanolactone, belonging to the neoclerodane class of diterpenes (Ortega et al., 1982). It is also now well established that salvinorin A is a highly selective and potent kappa opioid receptor (KOP) agonist (Roth et al., 2002). Salvinorin A is chemically and structurally unique from other hallucinogens, being the first known psychoactive diterpene and the first non-nitrogenous hallucinogen (Vortherms and Roth, 2006). Doses ranging from 200 to 600 μg produce profound hallucinations in humans that are also qualitatively distinct from the psychoactive effects produced by more traditional hallucinogens like lysergic acid diethylamide (LSD), mescaline, or psilocybin (Gonzalez et al., 2006; Siebert, 1994).

Hallucinogenic drugs comprise a distinct class of compounds categorized by their chemical structures and pharmacological actions. Direct investigation of the precise neurochemical mechanisms responsible for their psychoactive effects in humans is technically challenging. However, the study of the discriminative stimulus effects of hallucinogens using drug discrimination procedures in nonhumans is a particularly attractive investigative paradigm (Winter, 2009) because of the specificity of discriminative stimuli correlating with underlying cellular and molecular mechanisms of drug action (Holtzman and Locke, 1988; Colpaert, 1999). Drug discrimination investigations have reliably shown that hallucinogens with similar neuropharmacological actions tend to produce cross generalization, while compounds with distinct neuropharmacological actions fail to do so.

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At the present time, only five published studies have examined salvinorin A using drug discrimination procedures. Only one study trained animals to discriminate another hallucinogen (Li et al., 2008) and only two studies trained animals to discriminate salvinorin A (Baker et al., 2009; Butelman et al., 2010). Butelman et al. (2004) published the first study to demonstrate that salvinorin A substituted in rhesus monkeys (one male and two females) trained to discriminate the synthetic KOP agonist, U69,593 (0.0056 or 0.013 mg/kg; S.C.). The opioid antagonist, quadazocine blocked this substitution in all three animals, whereas the selective kappa antagonist, 5′-guanidinonaltrindole (GNTI) blocked these effects in only two of three animals. Willmore-Fordham et al. (2007) reported similar results in male Sprague–Dawley rats trained to discriminate U69,593 (0.56 mg/kg, I.P.). Baker et al. (2009) replicated these findings in male Sprague–Dawley rats trained to discriminate a lower dose of U69,593 (0.13 mg/kg, S.C.) or another KOP agonist, U-50,488H (3.0 mg/kg, I.P.). That study also showed full substitution with U69,593 and U-50,488H in rats trained to discriminate salvinorin A (2.0 mg/kg, I.P.).

In the only published study to examine salvinorin A in animals trained to discriminate a traditional hallucinogen, Li et al. (2008) trained four rhesus monkeys (two males and two females) to discriminate the serotonergic hallucinogen 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM; 0.32 mg/kg, S.C.) under a FR 5 schedule of stimulus shock termination. In these monkeys, DOM discrimination generalized to other 5-HT agonists, but failed to generalize to salvinorin A, ketamine, or PCP. Consistent with these findings, Butelman et al. (2010) recently reported that monkeys (three males) trained to discriminate salvinorin A (0.015 mg/kg S.C.) generalized to other kappa agonists, but did not generalize to psilocybin or ketamine. Moreover, salvinorin A discrimination was blocked by quadazocine but not the 5-$HT_2$ antagonist, ketanserin.

To our knowledge, there are currently no published studies on the effects of KOP agonists in animals trained to discriminate any of the dissociative hallucinogens. However, a few studies have tested the noncompetitive NMDA-receptor antagonists, phencyclidine (PCP), ketamine, and MK-801 for substitution in animals trained to discriminate synthetic KOP agonists. The results of these studies are somewhat equivocal. Shearman and Herz (1982) trained male Sprague–Dawley rats to discriminate between ethylketocyclazocine (0.32 mg/kg) or bremazocine (0.04 mg/kg) and saline in a food-reinforced discrimination procedure. They reported that PCP and ketamine substituted in some rats trained to discriminate bremazocine, but not in any of the rats trained to discriminate ethylketocyclazocine. In other studies, PCP failed to substitute in male Sprague–Dawley rats trained to discriminate the KOP agonist, spiradoline (U62,066; 3.0 mg/kg S.C.) in a discrete-trial shock-avoidance/escape procedure (Holtzman et al., 1991) or male Long Evans hooded rats trained to discriminate U-50,488H under a fixed ratio 20 schedule of food reinforcement. In contrast to these earlier findings, a recent study by Mori et al. (2006) reported that PCP, ketamine, and MK-801 all produced full substitution for U-50,488H in male Fischer 344 rats. These findings along with the recent discovery of salvinorin A's selective KOP receptor affinity warrant further investigations comparing the discriminative stimulus effects of salvinorin A with dissociative hallucinogens as well as other traditional hallucinogens. The primary aim of the current study was to assess the effects of salvinorin A in rats trained to discriminate either a prototypical serotonergic hallucinogen, LSD (Experiment 1) or the noncompetitive NMDA antagonist and dissociative hallucinogen, ketamine (Experiment 2).

## 2. Materials and methods

### 2.1. Subjects

Sixteen drug-naive male Sprague–Dawley® rats (Charles River Laboratories, Portage, MI) approximately 180 to 210 days of age at the start of the experiment were used. All rats were individually housed in polycarbonate cages within a climate-controlled animal facility and maintained on a 12-hour light/dark cycle with free access to water. Access to food was limited so that animals were maintained at approximately 85% of their free-feeding weights.

### 2.2. Apparatus

Behavioral training and test sessions were conducted using eight standard operant chambers (Med-Associates Inc., Georgia, VT) equipped with three retractable levers (left, center, and right) on the front panel, a food delivery mechanism above the center lever, and a 28-V house light located at the top of the rear panel. Experimental events and data collection were computer-controlled using MED-PC (version 4.0 for Windows) instrumentation and software. Lever pressing was reinforced with dustless precision food pellets (45 mg, product # F0021, Bioserv®, Frenchtown, NJ).

### 2.3. Drugs

Lysergic acid diethylamide tartrate (National Institute on Drug Abuse, Bethesda, MD) was dissolved in sterile 0.9% saline and administered via subcutaneous (S.C.) injection. Ketamine hydrochloride (Sigma Chemical Company, St. Louis, MO) was dissolved in sterile water and administered via intraperitoneal (I.P.) injection. Salvinorin A was generously provided by Mailman Research Center, McLean Hospital (Belmont, MA). Due to limited solubility, salvinorin A was initially dissolved in dimethylsulfoxide (DMSO) and then diluted with sterile water to 75% DMSO and administered via I.P. injection. All drugs were administered at a volume of 1 mg/ml and doses were determined based on the weight of the solid compounds.

### 2.4. Preliminary training

Prior to the lever-press training, subjects in both groups underwent two 60-minute sessions in which food pellets were delivered under a fixed time 60 s (FT 60″) schedule of pellet delivery in order to familiarize the animals to the stimuli within the operant chamber and the location of the food pellets. All levers remained retracted during these sessions. Animals were then trained to press the center lever for food pellets during a 20-minute session using a fixed ratio 1 (FR 1) schedule of reinforcement. Once lever pressing was acquired, a series of 20-minute errorless training sessions were conducted during which either the left or right lever was extended. Fifteen minutes prior to training sessions, each animal received an injection of either the training compound (Experiment 1: LSD, Experiment 2: ketamine) or its respective vehicle. For half the animals in each experiment, drug injections preceded sessions in which left lever responses were reinforced and vehicle injections preceded sessions in which right lever responses were reinforced. These conditions were reversed for the remaining animals in each group. An equal number of errorless training sessions were conducted for each animal under each training condition. Once animals were reliably responding under both the drug and vehicle conditions, discrimination training sessions commenced.

### 2.5. Discrimination training

During the discrimination training sessions, both the left and right levers were always present. Twenty-minute sessions were conducted once a day at approximately the same time of day 5 to 7 days per week. Drug and vehicle training sessions were alternated to include two or three of each stimulus condition per week, with the limitation that neither stimulus condition occurred for more than two consecutive days in a row. The programmed fixed ratio (FR) schedule of reinforcement was such that only a fixed number of consecutive responses would result in reinforcer delivery and an incorrect